

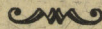
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**REPORT**  
of the  
**BLOOD TRANSFUSION ASSOCIATION**

**Concerning the Project for Supplying Blood  
Plasma to England, Which Has Been Carried  
on Jointly with the American Red Cross from  
August, 1940, to January, 1941**



**Narrative Account of Work  
and  
Medical Report**



*January 31, 1941*

**BLOOD TRANSFUSION ASSOCIATION  
2 EAST 103D STREET  
NEW YORK, N. Y.**

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### PART I

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NOTE: It was not deemed necessary to print Appendices A, B, C, D and E, referred to in the report and containing voluminous statistical, financial and other details of the work, together with the forms used, and these have therefore been bound separately.

## PART II

### Medical Report Submitted in Behalf of the Board of Medical Control by the Medical Supervisor of the Blood Plasma Division, the Chairman of the Board, the Chairman of the Blood Plasma Committee, and the Assistant to the Board, Blood Plasma Division:

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**Report by the Blood Transfusion Association Concern-  
ing the Project for Supplying Blood Plasma to England,  
Which Has Been Carried on Jointly with the American  
Red Cross from August, 1940, to January, 1941**

**Preliminary Statement**

In accordance with the request to make the essential information gained by this experiment immediately available for purposes of national defense, this report is submitted in advance of entire completion of the work. Because of this factor and the pressure of time under which it has been prepared, the medical portions of the report, and the statements based thereon, should not be understood as any attempt to express final scientific conclusions, but are intended only to give in clear form the best practical information and suggestions which are immediately possible in a field which is still in the experimental stage and subject to rapid development.

The financial report will be supplemented later by a fully audited statement of the entire project, certified by Messrs. Haskins & Sells.

The Blood Transfusion Betterment Association is in process of changing its corporate name to the "Blood Transfusion Association" and has been described in this report by the latter title.

**Outline**

The report consists of:

PART I

A narrative account of the project with a discussion of the difficulties and problems encountered, the lessons which these have taught applicable to future work in this field, and certain recommendations. Comment, conclusions and recommendations in this part of the report which deal with medical or scientific subjects are based upon the medical report [Part II hereof]. Appendices separately bound are submitted covering:

A. Report as to the administrative setup, procedures, forms, etc.

B. Report of the numbers of donors enrolled, of blood taken and plasma produced and the disposition thereof.

C. Financial statement based upon actual figures to December 31, 1940, and estimates to the expected completion of the project at about January 31, 1941.

D. List of all individuals and organizations assisting in the project.

E. Estimate, submitted pursuant to request of American Red Cross, of expenses involved in equipping and staffing a laboratory for the bacteriological control of plasma.

## PART II

A full medical report by the Medical Supervisor of the Blood Plasma Division, the Chairman of the Board, the Chairman of the Blood Plasma Committee, and the Assistant to the Board, Blood Plasma Division, which is bound herewith.

## PART I

### Narrative Account of the Work, Problems Encountered and Conclusions Therefrom

#### THE ASSOCIATION

The Blood Transfusion Association is a non-profit membership corporation, organized in 1929 for the principal purpose of improving the supply of blood for transfusion purposes in New York City. To this end it has since conducted a Blood Donor Bureau, and it has also among other activities more recently supplied a special high-titred serum for matching blood groups. Such surplus funds as the Association has had above a modest reserve have been devoted to the financing of research in the transfusion field, including the development of blood substitutes. The medical and research work of the Association has been throughout conducted under the advice of a Board of Medical Control including the leading hematologists and transfusionists in this City.

#### RESEARCH IN PLASMA FIELD

Part of the research program which the Association financed last year dealt with blood plasma. Such work was carried on by Dr. John Scudder and Dr. Charles R. Drew, and was done at Presbyterian Hospital in conjunction with certain work in the same field simultaneously in progress at the Rockefeller Institute. These research workers also, of course, kept in close touch with similar work done elsewhere.

#### ORIGIN OF PROJECT

As a result of all of this, it was felt in June, 1940, that knowledge of blood plasma had reached a point at which it might be used effectively for saving life in the warfare then going on in the low countries and France, as information had been received that casualties were high, both in the armed forces and among the civilian population, as a result of shock from continued bombing, strafing and exposure. The idea of shipping plasma to France and England was suggested by Dr. Scudder to the President of the Association in June, 1940.

On June 12, 1940, the President called a joint meeting of the Association's Trustees and Board of Medical Control to consider



this general subject, and invited Dr. Alexis Carrel, who had recently returned from France, as well as experts in the field representing the Army, Navy, National Research Council, Rockefeller Institute, New York Academy of Medicine, and the large pharmaceutical and biological companies, including Burroughs-Wellcome, Lederle Laboratories, Sharp & Dohme, Parke-Davis and Squibb.

At the meeting Dr. Carrel pointed out the great need and usefulness of plasma for war shock cases in France; Captain Kendrick of the Army spoke favorably as to the efficacy of plasma; and Dr. Scudder presented some results of the recent research.

It was the sense of the meeting that, although the use of plasma was still in an experimental stage, enough knowledge was available to justify an effort at quantity production to save lives of war casualties in the emergency then existing.

Immediately following the joint meeting, a special meeting of the Board of Trustees of the Association was held. The Trustees felt that the project should be submitted to the American Red Cross as the dean of humanitarian agencies in war relief work, and it was voted to sponsor the production and shipment of plasma to the Allies, and to appropriate \$15,000 from the Association's funds to start the project immediately, provided the Red Cross was willing to cooperate.

It was also decided to place at the disposal of the Red Cross all technical knowledge and experience which the Association had gained in its research for such use as the Red Cross might wish to make of it.

Mr. Voorhees, one of the Trustees, was designated to assist the President, Mr. Bush, and the Chairman of the Board of Medical Control, Dr. Stetten, in formulating the program and presenting it to the Red Cross.

It was also voted to make an immediate preliminary survey of hospitals in Greater New York which might be willing to cooperate in such a program, and also that a similar survey be made as to the extent of cooperation which might be possible from the major commercial laboratories.

Since there was a lack of adequate data concerning preparation of dried plasma and its efficacy, it was decided that liquid plasma would be utilized for the time being.

Pursuant to such authorization of the Board of Trustees, a tentative memorandum, with the preliminary information then available and rough estimates of what might be possible in this field, was

prepared and presented to the Red Cross by the President, Dr. Stetten and Mr. Voorhees. Such proposals contemplated cooperation in the work by various voluntary hospitals in New York.

By the time of such meeting with the Red Cross, France had fallen, and England, with the impending threat of invasion, represented the center of immediate need.

Investigation promptly disclosed that statistics based on casualties in the last war would be of little use in estimating requirements for plasma transfusions, and that the quantities produced would have to be worked out largely without the benefit of past experience.

A committee consisting of Drs. Corwin, Drew and Scudder, was appointed by the President to make recommendations as to what equipment and personnel might be made available by the cooperating hospitals and as to what might have to be supplied by the Association. This committee submitted a report dated July 1st covering the subjects of personnel, requisite floor space, supplies, preparation of equipment, serology, bacteriology, supervision, routines for non-centrifuge technique for the preparation of pooled plasma, and criteria for the selection and protection of voluntary donors.

It was also ascertained that the Surgeons General of the Army and Navy had taken up the subject of blood transfusions and treatment of shock with the National Research Council, and that the problem had been entrusted by the latter to a special Committee of which Dr. Walter Cannon of Boston was the Chairman and in which Dr. Cyrus C. Sturgis of Ann Arbor, Michigan, headed a Subcommittee on Blood Substitutes. Contact was, therefore, at once made with Dr. Sturgis to obtain his views and to coordinate any program with the work of his Subcommittee.

#### PROGRAM AGREED UPON

After some consideration of the possibilities of larger production, it was finally proposed to the Red Cross that the initial program should be limited to an expenditure of about \$40,000 since the first work must be essentially of an experimental character. In addition to the contribution of \$15,000 which the Association had voted to make, as above noted, the American Red Cross was therefore asked to advance \$25,000 and to conduct the campaign for donors through its Chapters in the Metropolitan Area. This proposal in substance was accepted by the Red Cross, which later

added to its appropriation \$5,000 more for special purposes, and the Association also added \$5,000 further from its treasury for research.

#### PROJECT BEGUN

Promptly upon the completion of such arrangements, the American Red Cross and the Association commenced the joint program in August, 1940.

The Association secured office space in the New York Academy of Medicine Building at 2 East 103d Street, such space being donated by the Academy.

The assistance of six of the leading voluntary hospitals was initially enlisted to take the blood and to prepare the plasma, as follows: Presbyterian, New York, Memorial, Mount Sinai, Post-Graduate, and Long Island College.

#### APPOINTMENT OFFICE

The Association and the New York and Brooklyn Chapters of the Red Cross mutually agreed that all public appeals for donors should ask that response be made directly to the office at the Academy of Medicine, which would be constituted as a central bureau to make appointments for donors at all of the cooperating hospitals.

A complete administrative outline for the setup and telephone arrangements of this office had been previously prepared and charted. Pursuant to this plan the telephone number given to the public was separate from the number used for other administrative and routine calls. The former was not connected through a switchboard, but the outside lines, under consecutive numbers, were connected directly to ordinary telephone instruments so that the calls would come in automatically in the first instance to the appointment clerks, without a switchboard bottleneck.

The appointment clerks were trained to give a full explanation in answer to all inquiries concerning the object of the program, the nature of plasma, the need, the method of taking the blood, the effects of giving blood, etc. Many persons who telephoned to make inquiries because of interest in the program who were doubtful or timid about giving blood were thereby persuaded to do so.

#### ADMINISTRATIVE STEPS

Standardized procedures and forms for confirming the appointments to the donors and the hospitals were adopted; and appropriate card-indices, records of donors and of appointments kept and lapsed were set up and filing systems installed. A system for financial control and accounting was established as promptly as possible, and arrangements were made with Messrs. Haskins and Sells to audit the work.

Forms of releases to the Red Cross, the hospitals and the Association, to be signed by donors, were prepared, and appropriate instructions were prepared and given the hospitals as to administrative steps and medical and technical procedures for safeguarding the donors, maintaining sterility of the plasma and standardizing the process as far as possible.

The plan contemplated utilization of the hospitals to supply laboratories and other facilities, equipment, and the technical personnel requisite to take the blood, preserve it and process it into plasma. This was necessary as the hospitals contained the only laboratories, equipment and personnel available. It was recognized that such a plan entailed the disadvantage that taking the blood and preparation of the plasma would be carried on in several different widely scattered laboratories, with consequent difficulty of control, but this was unavoidable. The hospitals and most of the members of their staffs, including doctors and nurses, volunteered their services. This help made the program possible, but the volunteer nature of it, and the large numbers of persons consequently participating, of course increased the difficulty of controlling technical procedures.

A detailed report as to the administrative setup of the program, with an organization chart and copies of all forms utilized, is submitted as Appendix A hereto, and is bound separately.

#### MEDICAL CONTROL

The Association, having agreed with the Red Cross to assume responsibility for taking the blood and preparing the plasma, arranged through its Board of Medical Control, of which Dr. Rhoads was then acting as Chairman during the absence on vacation of Dr. Stetten, to supervise and check as closely as possible the work done in the several hospitals. In this work the Board was aided by Dr. Scudder. It was found at an early date that it was

necessary, both for adequate control and for study of improvements in the procedures, to engage a full-time Medical Supervisor, as Dr. Scudder could devote only part of his time to the work of supervision on account of his hospital and research duties. Dr. Drew, who with Dr. Scudder had previously been associated in the conduct of the research work on which the program was in part based, was recalled in September from teaching duties in Washington which he had just assumed, and was made a full time Medical Supervisor. Dr. Stetten, on his return early in September, resumed and continued thereafter his duties as Chairman of the Board of Medical Control, and Dr. Rhoads became Chairman of a Blood Plasma Committee created by such Board.

#### THE COOPERATING HOSPITALS

Before the experiment began, some twenty hospitals had evidenced to the Association their willingness to cooperate. However, detailed studies of the respective hospitals' facilities and personnel available for the task made it seem desirable to limit the program at first to the six institutions above named, particularly as the doctors in charge of this work in most of these hospitals were members of the Board of Medical Control and could, therefore, readily test the procedures and exchange information. Such limitation of the number of hospitals in which work was almost immediately begun was also indicated as desirable because of initial difficulties in procuring donors, as a result of which the hospital capacity was for some time more than adequate for the supply of blood.

Purchases of refrigerators and other equipment as needed were promptly made by the various hospitals at their own cost, and the Association supplied equipment of a character which the hospitals would not require for their ordinary work, such as special glassware and, in certain instances, centrifuges where these were not already available. The order and dates on which these hospitals started work were as follows:

	1940
Presbyterian Hospital .....	Aug. 15
Mount Sinai Hospital .....	Aug. 16
New York Hospital .....	Aug. 27
Long Island College Hospital (Brooklyn)	Aug. 27
Memorial Hospital .....	Sept. 4
New York Post Graduate Hospital.....	Sept. 4

Later the following hospitals also joined in the work:

	1940
Lenox Hill Hospital .....	Sept. 23
Hospital for Joint Diseases .....	Oct. 1
Jewish Hospital of Brooklyn .....	Dec. 2

#### PUBLICITY AND CAMPAIGN FOR DONORS

Since the New York and the Brooklyn Chapters of the American Red Cross, which were under separate direction and had separate publicity departments, were both asked by National Headquarters to assist in the program, it was initially necessary for them to coordinate such dual efforts and determine their respective spheres of activity, particularly in publicity. Cordial cooperation on the part of the publicity representatives of the two Chapters went far to solve problems which under such a divided organizational setup might well have proved serious.

Early in September executive charge of the work of obtaining the donors by the New York Chapter was taken over by Captain Scully and was carried forward with the greatest effectiveness and a most helpful spirit of cooperation. In this work he was assisted by Mr. Sinclair of the Publicity Department, and most favorable publicity was obtained.

The work in the Brooklyn Chapter was under the competent direction of Colonel Bigley, the Executive Director, who was effectively assisted by Miss Coxson and by Mrs. Warner, the latter being in charge of publicity.

Through the publicity avenues open to the Red Cross, this joint work by both Chapters resulted in obtaining adequate radio time as well as dignified and effective publicity in the metropolitan newspapers and national magazines.

As a result of all this, the flow of donors gradually increased, so that at the peak of the program appointments for 1,200 to 1,300 donations per week were being made at the hospitals.

Close liaison was maintained at all times between the Association and both the New York and Brooklyn Chapters, and a spirit of the friendliest cooperation prevailed throughout.

#### REGULATING THE SUPPLY OF DONORS IN RELATION TO HOSPITAL CAPACITIES

Such close cooperation between the New York and Brooklyn Chapters of the Red Cross and the Association was especially



necessary in order to keep some approximate balance between the flow of donors and the capacities of the hospitals, a subject which presented for a time a serious problem. In the early weeks the number of prospective donors was unpredictable, and the supply varied widely from time to time. There were periods during which an insufficiency of donors discouraged the hospitals and tended to diminish the interest of their volunteer doctors and nurses. On the other hand, prospective donors' interest was lost if an early hospital appointment could not be made, but hospital capacities under the volunteer program were inelastic and could not be quickly expanded to meet temporary peaks.

The principal credit for progress in solving this very real problem belongs to Captain Scully, who after several weeks became able to gauge with considerable accuracy the effect of particular publicity efforts in producing donors and thereby to keep the supply more nearly in balance with hospital capacities.

#### DONOR RESPONSE

It is believed that the knowledge gained in this experiment as to the attitude of possible donors will itself prove of great value in any future program. Prior to this experience, there was no method of estimating how donors would respond to appeals for mass volunteer blood donations. Opinions varied from an expectation of an overwhelming response to beliefs that there would be great difficulty in obtaining any considerable number because of apprehension on the part of the public, and the revulsion which many people feel at the sight of blood or even the thought of bleeding.

Experience proved that the truth lay perhaps midway between these estimates. Initially there was great difficulty, but, as the public became educated, there grew a genuine enthusiasm on the part of many to make this very personal gift for war sufferers. It is believed that the program demonstrated that blood can be obtained on a volunteer basis in large quantities, but that this can be done only by intensive and continued newspaper publicity and radio appeals.

It is also believed that the greatest difficulty in obtaining donors arises from the necessity of having them come to hospitals or some other central place, instead of its being possible to go to many of them at plants, large offices and stores to take the blood there. Certain suggestions on this subject for future programs are set forth later in this report.

#### DEVELOPMENT OF LIST OF DONORS

A very important aspect of the work has been the development of a list of about 17,000 donors who have been educated to give their blood and whose continued interest and cooperation may be expected in large part in any future program. The names, addresses, ages, telephone numbers and blood groups of these donors, and the fact that they were disease-free are all known. This supplies an invaluable nucleus for any new program.

#### WORD FROM ENGLAND AS TO TERMINATION OF NEED

It had been planned further to expand the program to other hospitals when early in November a letter was received by the Association from England, saying that the maintenance of the existing supply of donations to about February 1, 1941, would be sufficient, and that thereafter England could take care of the situation herself. Because of this no further efforts were made to equip additional hospitals (with one exception where preparation was already in progress).

The bleeding schedule, accordingly, continued at a rate of about 1,200 per week until December, when final advices from England were received that bleeding could stop about January 1st. The program was therefore brought gradually to a close, and the last blood was taken January 17, 1941.

#### PLASMA PRODUCED

Detailed statistics as to appointments, donations and plasma produced are set forth in Appendix B hereto, which is bound separately. The total appointments made for donors were 18,861. The total donations actually taken were 14,556. The total plasma saline solution produced and either passed by the Association as satisfactory, or on hand and believed to be satisfactory subject to the completion of tests, is conservatively estimated to be approximately 5,500,000 cc, or 5,500 liters. (The exact amount can of course not be determined until the completion of tests, and the total figure may somewhat exceed this amount.)

#### FINANCIAL

Detailed figures are given in Appendix C hereto—bound separately—and a very general statement is all that is, therefore,

necessary here. The Association paid over in full the \$15,000 appropriation which it had made. The Red Cross paid over in full its \$25,000 original appropriation. It has not yet been necessary to ask for the additional \$5,000 appropriation by the Red Cross above referred to. The Association's own additional appropriation of \$5,000 for research has been approximately fifty per cent utilized, and expenditures chargeable against this are continuing.

Arrangements for the division of any unexpended balance of the appropriations for this project were incorporated in the agreement between the Association and the Red Cross last summer, and such adjustment will, of course, await the final figures and audit.

Exclusive of special expenses for research from the separate fund mentioned, the total cost of the entire program based on actual figures to December 31st and estimates to the completion of the work, will it is believed be about \$40,500.00.

Since this amount is in part estimated, and subsequent figures mentioned herein are partially based upon the same estimate, all of the figures must be understood to be merely approximations which will be subject to revision on final audit. The present figures will, however, serve to give a general idea of the cost of plasma produced by a volunteer project of this kind.

Even if the capital charges for equipment purchased are treated as written off entirely as an expense during the project, the cost per liter of plasma saline solution produced and passed under the Association's tests as satisfactory, or which is on hand and, subject to completion of tests, is believed to be satisfactory, is about \$7.35. This is equivalent to a cost per liter of plasma without saline addition of about \$14.70. Such costs, excluding capital charges, are \$6.35 and \$12.70 per liter, respectively. The above figures of \$14.70 or \$12.70 for the cost per liter of plasma without the saline addition compare with a commercial market price per liter of plasma of \$138.00 (Cutter Laboratories) and a non-commercial price of \$70.00 from the Deutsch Serum Laboratories of Chicago, which is a non-profit organization, and which it is understood gets blood at very low cost.

It should also be noted that the fact that the commercial market price for plasma is held at the figure above given is possible only because the blood for such plasma is procured in the far west at a fraction of the rates prevailing in New York for professional blood donations.

At such commercial market price, the value of the plasma produced for England, based upon actual figures to December 31st and estimates thereafter, is approximately \$400,000. This plasma has, therefore, been obtained through the volunteer program at a cost, including capital charges, of slightly more than 10%, and, excluding capital charges, of less than 9% of its commercial value, this extremely low relative cost being due, not only to the donation of the blood, which is of course the largest item, but to the volunteer character of the work throughout.

#### VOLUNTEER NATURE OF THE PROJECT

All officers of the Association served without pay, as did the Director and the doctors on the Board of Medical Control. Dr. Scudder also contributed his services without compensation from the Association. The Association's only paid medical staff has consisted of the full-time Medical Supervisor who, however, made a great personal sacrifice in order to return to this work.

Some of the services even of the office staff were of an entirely or partially volunteer nature, and the only paid force consisted of the stenographic, clerical and appointment staff who, however, worked with great devotion to the cause, and put in long overtime hours without extra pay.

All of the extensive research work done by the members of the Board of Medical Control and their staffs was, of course, upon a volunteer basis.

The hospitals contributed equipment, facilities, and salaried personnel without charge to a substantial extent; the balance of the hospitals' costs of doing the work being met by a flat payment per liter of plasma, the amount of which was fixed by the Association as equitable. The hospitals enlisted many volunteers from their medical staffs to take the blood and process the plasma. Also nurses in large numbers, after completing their paid work-day on their regular hospital assignments, contributed their services for extra hours in the project. The work of the hospitals was also very effectively supplemented by Red Cross Grey Ladies, who were made available by the Chapters to serve as receptionists and hostesses, and who gave help and confidence to the donors and generally assisted the hospitals in all ways possible. Also, certain Red Cross staff assistants were assigned to the central appointment office, where they were most helpful.

The entire program has, therefore, represented an outpouring of humanitarian effort of a novel kind and on a large scale. It has been a spontaneous expression of a deep public sympathy for, and of a keen desire to lend help to, war sufferers in England. In more than one sense it has been unique among relief projects: It has represented a gift which was literally a part of each donor; and has been, therefore, of a more personal nature than any contribution of money or articles could be. It has also represented a form of aid which England could not buy or otherwise obtain, since the cost would have been prohibitive even if the blood in sufficient quantities had been obtainable on a commercial basis. It is felt, therefore, that the Red Cross, the Association, and the cooperating hospitals, may look with justifiable pride upon the record which has been made.

While the system of taking the blood and processing the plasma in the voluntary hospitals is not ideal—because the highly technical work was scattered instead of being centralized, and was done by many volunteers instead of a relatively small paid staff—it was only by the hospitals' generous cooperation that this work for England could have been done at all. Through their chief doctors, many of whom were members of the Board of Medical Control of the Association, the hospitals also furnished a sum total of medical knowledge which was a prerequisite to the conduct of the program in the first place, and which has constantly developed new knowledge on the subject.

The project has been, not only an experiment in the mass collection and handling of blood and in the widespread use of a relatively new extension of blood transfusion therapy, but also a concrete demonstration of the ability of large numbers of volunteers to integrate themselves unselfishly into a working unit to carry on a program which required very close coordination, considerable technical skill and large sacrifices of time, money and effort on the part of each individual.

Approximately two thousand volunteer workers have taken part in the portion of the work carried on by the Association and the hospitals, which, of course, does not include the very extensive work done by the Red Cross itself.

A full list of those who contributed their services in the portion of the program for which the Association assumed responsibility, together with the corporations, agencies and individuals contribut-

ing to or participating in the work, has been compiled as Appendix D hereto and is bound separately.

However, mention should be made here of a few organizations and individuals rendering outstanding service, such as:

The New York Academy of Medicine, which donated office space and supplied furniture and other facilities.

Messrs. Haskins & Sells, who did all the accounting work for the project without charge.

Messrs. Pettit & Reed, who without charge supplied refrigerated storage place for the plasma throughout the project, remodeled the storerooms so that the plasma from the respective hospitals might be kept separate, provided refrigerated trucking for the plasma from the various hospitals to the warehouse and, through their Mr. Edward Levy, took complete and wholly satisfactory charge of the records of shipment.

Mr. Morris M. Davidson, who served as a full-time volunteer as Director of the Blood Plasma Division in charge of the administrative work of the project.

And the following who were responsible for heading the medical and technical side of the work:

Dr. DeWitt Stetten, Vice-President of the Association, who assisted in launching the program, and is the Chairman of the Board of Medical Control.

Dr. C. P. Rhoads, Medical Director of the Memorial Hospital, who served during the summer as Acting Chairman of the Board of Medical Control during Dr. Stetten's absence, and throughout as Chairman of the Blood Plasma Committee of the Board, and who also participated in the work of Mr. Folsom hereinafter mentioned.

Dr. John Scudder, who first proposed the shipment of blood plasma to the Allies, helped to get the project started, served as Assistant to the Board of Medical Control, and who also acted as Director of the work at Presbyterian Hospital.

Dr. Charles R. Drew, who, as Medical Supervisor, had charge of coordinating the medical aspects of the program, establishing uniform records, standard equipment and criteria in order to insure the safety of the final product.

Dr. Frank L. Meleney, who served as Bacteriological Consultant, and his associate in such work, Miss Balbina Johnson, who together did a great volume of work of an indispensable character in making all bacteriological tests of the plasma.

Dr. Nathan Rosenthal, Director of the Department of Hematology at Mount Sinai Hospital, who organized a large hospital unit which did over 3,000 phlebotomies and which both in the quantity and quality of its work was throughout outstanding.

Dr. Lester J. Unger, Director of the Plasma Laboratory and Blood Bank at the Post-Graduate Hospital, who directed the collection of blood and the processing of plasma both at that hospital and at the Hospital for Joint Diseases, and who carried out important experiments for improvement in techniques.

Dr. Ralph G. Stillman, Director of Laboratories at the New York Hospital, who took over the responsibility for the entire program there.

Dr. E. R. Marzullo, who directed the program at the Long Island College Hospital in Brooklyn.

Dr. Perry J. Manheims, who directed the work at the Lenox Hill Hospital.

Dr. Alexander S. Wiener, who established at the Jewish Hospital a second center for drawing blood in Brooklyn.

Dr. William Thalhimer, Director of Manhattan Convalescent Serum Laboratory, who furnished the facilities of his laboratory, and who worked assiduously in the experimental program.

In addition to the above mentioned services of these doctors, almost all of them made important contributions to the research work connected with the program.

Mention should also be made of the contribution of Mr. Theodore R. Folsom, a physicist on the staff of Memorial Hospital, who created a new apparatus for drying plasma.

#### **Miscellaneous Problems Which Have Been Encountered, and Suggestions Derived from Them for Any Future Program**

##### **FEARS OF DONORS**

Many persons who were interested feared the operation involved in giving blood or the effects of the loss, or both. It was therefore found important to have receptionists or hostesses to greet the donors, excuse delays if necessary, give explanations desired, allay fears, and generally to look after their comfort after the donation. This was done for the most part by Red Cross Grey Ladies. This phase of the work is especially important in securing repeat donations.

##### **CARE OF DONORS**

Pursuant to the policy stated by the Chairman of the American Red Cross at the inception of the program, every possible precaution has been taken to protect the donors. The Board of Medical Control considered this subject at once, and the hospitals were instructed, in addition to a physical examination, to determine the donor's blood pressure, temperature and hemoglobin. No person was permitted to give if in a condition such that harm might have resulted. To protect elderly persons offering blood, it was decided as a matter of fixed policy not to accept donations from anyone more than sixty years old. Donations from minors were also declined, both for their own protection and because they were not in a position to execute valid releases from liability.

For the protection of donors, rigid instructions were given to all hospitals that under no circumstances was it permissible to "cut down on the veins"—that is, to make an incision to expose the vein.

To guard against a sensation of weakness on the part of donors during or after the giving of blood, donations were in most instances taken while the donor was lying down. The hospitals furnished milk, egg-nog, whiskey or other light refreshments to the donors following bleeding, and a reasonable rest period after the donation was prescribed.

Some donors felt faint after giving their blood, and there were a few instances of donors fainting. Some of such after-effects were thought to be psychological in origin, although nevertheless real. There were isolated instances, in spite of all precautions, of donors fainting after leaving the hospital, where they left without notifying the hospital authorities that they were not feeling all right, but in all instances where there was any indication that donors were experiencing any weakness after giving blood, care was at once given to them by the medical staff of the hospital.

Due to all of these precautions, the outstanding fact in this aspect of the work is that, of the many thousands of donors who gave their blood, no instance was reported of any serious or prolonged after-effects of the donation, and there was no report of any infection developing.

In any future program, continuation of these methods of care and personal solicitude for the welfare of donors is believed to be of primary importance, and it is thought that this will be of great help in securing repeat donations from the same persons.

Tests to make certain that the donors were not syphilitic were performed after the blood was taken, and any contaminated blood was discarded. Where disease was discovered, the donors were advised by the hospital which took the blood that a condition had been found which made it important for them to consult a physician.

#### BLOOD GROUPING

It was found that the donors were very interested in knowing their blood group—sometimes referred to as blood type. The work of grouping the blood was not absolutely essential to the production of plasma, and was done primarily as a courtesy to the donors, and cards were later sent by the American Red Cross certifying the blood group to which they belonged.

#### BUTTONS FOR DONORS

The Association recommended early in the experiment that a lapel button or other similar insignia be given to donors, but the Red Cross decided against this and in favor, for this project, of the personal certificate above referred to giving the donor's blood group. In England's volunteer blood giving system, badges are used for repeat donors, who however must pay for them. It is believed that, if a large body of donors is to be built up who will give repeat donations, an appropriate button or other suitable insignia would be most helpful in stimulating the interest not only of the donor who wears it but of others who will notice it and inquire about it.

#### PEAK LOADS OF CALLS FOLLOWING BROADCASTS

One difficulty presented was the great number of proffers of blood which would immediately follow a radio appeal, making it impossible at times to answer all calls. This was partially met by having an excess reserve number of direct lines and telephone instruments available in the series of consecutive telephone numbers previously described, and by having all office employees, whatever their regular function, trained to answer such calls. The appointment clerks were also instructed at such times to obtain merely the names and telephone numbers of the prospective donors, and to inform them that the appointment office would call back and

make the appointment as soon as the peak load was passed. In view of the value of the blood as compared with the administrative cost, it was felt that every effort should be made not to lose offers of blood through inadequate office facilities or personnel.

#### LAPSED APPOINTMENTS

Another problem concerned the failures of donors to keep appointments. Experience throughout indicated an average of about 15% who failed to appear. Since this ran fairly uniformly, allowance was made for this in making the appointments.

#### NEED OF DEVELOPMENT OF NEW PUBLICITY MATERIAL

A conspicuous handicap under which the Red Cross labored in the program for procuring donors was the lack of pictures and publicity stories from England of the use of the plasma, or reports or letters from persons in high official position which could be used for publicity purposes to show its usefulness in saving life. The story of the project itself was a very simple one, and once told there was little which could be added to create new publicity material. A cable from one of the English Army Medical Officers, requesting that an amount of plasma, the production of which would have required 20,000 blood donations, be shipped in thirty days, was the most helpful publicity material received from England. However, it would have been too much to expect that with the sufferings which the English people were undergoing during these months they could find opportunity to create publicity material of this character. In any continued program, however, such pictures and stories to keep the public plasma-conscious would be most useful.

#### TRAINING OF PERSONNEL

In the belief that a national defense program for the production of plasma might be soon needed, the Association has kept constantly in mind the development of medical and technical as well as administrative personnel.

The New York and Brooklyn Chapters of the Red Cross have also developed personnel with invaluable training for a campaign to procure donors elsewhere.

A limited number of experienced personnel is therefore now available to the Red Cross or the Army or Navy, as a result of the program just closing, for use as needed.

#### **Research Aspects of the Program, Medical Problems and Relation to National Defense**

It has been throughout borne in mind by the Association and the doctors connected with it in this work that the knowledge gained might be useful for national defense as well as for general civilian peacetime purposes. Attention has therefore been focused throughout upon the research aspects of the program and the development of new and improved techniques, and it was to this end that the Association made the extra appropriation of \$5,000 for research.

Not only has research been carried on as part of the program, but in a very real sense this entire enterprise was in effect itself a research project in the possibilities of mass production of plasma from volunteer donors.

Except for the urgency of the need, it would have been more normal to have spent many months in experimentation before attempting large scale production, and we were on this account, as well as because of the newness of the entire field, prepared to experience the unavoidable mistakes and hard lessons of any pioneering program.

But the interest in such a large production program itself served to stimulate research and improvements, and the experience gained in mass production also brought much necessary knowledge. Such forced hothouse growth of the plasma-plant has brought it nearer to maturity in these few months than would have otherwise been possible, even though there were certain limitations which the conditions of this program placed upon research work, to which reference is hereafter made.

Based upon the experience so gained, various proposals for continued experimentation and work have been formulated and are herein discussed, and certain suggestions relative to plasma production for national defense are made.

The medical and scientific aspects of the work and the problems connected therewith are, of course, more scientifically and completely considered in the medical report, which forms Part II hereof, but it may be helpful in the present portion of the report,

written by laymen primarily for laymen, to include a brief mention of some of them based on the information contained in the medical report.

#### **LIAISON WITH THE NATIONAL RESEARCH COUNCIL AND THE PLASMA WORK CARRIED ON IN ENGLAND AND CANADA**

Pursuant to the general purposes above stated, the Association, even before the institution of the project, sent one of its doctors to Ann Arbor to discuss the plan with Dr. Cyrus C. Sturgis, as the head of the Subcommittee on Blood Substitutes of the National Research Council. Thereafter contact was maintained with Dr. Sturgis to make the project here a helpful part of the work of his subcommittee, and he twice visited New York at the invitation of the Association to study the work.

Contact has also been preserved throughout with the similar program being carried on in Canada, where for approximately a year some plasma and serum have been produced under Dr. Charles H. Best, who has developed valuable knowledge upon the subject, although the number of donors has until recently been relatively small compared to the scope of the project here.

The Association has also obtained such information as was possible about work in this field being carried on in England, although conditions have made a full interchange of information difficult. Prior to the retreat from Dunkerque, the Blood Transfusion Service of the English Army had created numerous mobile transfusion units carrying preserved whole blood for field transfusion service. Apparently almost all of these units were lost on the retirement of the Army from the Continent. The blood for this service had been in large part obtained by mobile bleeding units, transported in lorries, which were set up for a day at a time in different localities. After Dunkerque, we now learn that attention of the Army Transfusion Service was directed to plasma production approximately simultaneously with the starting of our work here. The mobile bleeding units above mentioned were then utilized or others were created for this purpose. Several central laboratories for processing plasma were set up, and a modified type of cream separator has been used, together with certain filtration processes, the latter however apparently not having been found entirely satisfactory.

The proposal herein made for a model or so-called "pilot" laboratory for processing plasma in New York, which had been



worked out here without our having knowledge at the time of the English work, was found closely to approximate methods employed by the doctors there.

The spur of the acute need in England, the unlimited supply of donors available due to war psychology and the concentration on the work of the Transfusion Service of the Army, including a paid staff in the laboratories, have, of course, accelerated developments. But it should be pointed out that in the work in Canada and in England, satisfactory knowledge of what is being done can only be obtained by personal observation. This is so because the doctors engaged have been too busy to stop for scientific writing and because knowledge of the subject has been in too fluid, incomplete and experimental a stage to justify final scientific conclusions. It was for this reason that, realizing how helpful knowledge of the concurrent work being done in England would have been during recent months in the program here, consideration was given to the subject of sending trained observers abroad. If this is not done, other means for continuously observing and reporting promptly upon progress and experience there should be adopted to avert duplication and delay here. *At present there is no adequately organized liaison existing, and the importance of better organization for this purpose in saving time, money, blood and perhaps avoiding serious disappointments can hardly be overstressed.*

#### CERTAIN LIMITATIONS OF THE PROGRAM RELATIVE TO RESEARCH

While it has been noted that the stimulus of the need, and the interest created, tended to foster productive research and to teach many practical lessons for any future program in this field, in another sense the necessities for such mass production have been a handicap. It was, for example, necessary to standardize and freeze the processes in order to maintain the output and to keep the costs in line with expectations. The uncertainty of the duration of the need of American plasma for England made it unjustifiable to make the substantial capital expenditures which would have been requisite fundamentally to alter the production process in accordance with knowledge acquired during the program, especially as an intimation was received from England early in November that the aid would not be necessary after the 1st of February.

Another aspect of the program which retarded important research lay in the moral obligation to ship the entire product to England to comply with the purpose of the project and the expectation of the donors, although it would have been most valuable to have diverted substantial quantities of blood or plasma for further research, including tests of therapeutic values. Only a very small amount of plasma was therefore available for research purposes, and this was procured from donors who gave it for this special object.

Due to these factors, and the circumstance that England's need for the supply of plasma from America has terminated earlier than might have been anticipated, *the project for England has ended before certain vitally important questions which have arisen affecting any work in this general field have been answered. It is believed that the continuation of intensive efforts to seek such answers is of first importance as a prerequisite to the production of large quantities of blood substitutes for purposes of national defense.*

#### DIFFICULTIES ENCOUNTERED RELATIVE TO MAINTAINING STERILITY OF THE PLASMA

At the inception of the project, the rules of the National Institute of Health—a Division of the United States Public Health Service—concerning the preparation of biologicals were applied to all work done. At the invitation of the Board of Medical Control, representatives of the Institute came to New York and passed upon and informally approved the bacteriological techniques to be adopted. It was thought that such precautions would be a thorough insurance against difficulties from contamination.

As an additional precaution, a preservative was added to prevent bacterial growth. But it was later found that, where plasma was kept over long periods, the concentration of such preservative was not in all cases sufficient to preserve sterility.

At an early date in the work it was realized that the prevention of contamination of the plasma was a more serious problem than had at first been thought, particularly as close supervision was difficult since the work was carried on in various separate hospitals and by a large number of volunteer doctors and nurses. Later, we were informed that the English were themselves having difficulties in maintaining the sterility of their own plasma.

In the first few weeks of the project there was a loss of substantial amounts of plasma while in course of preparation, which was found by certain of the hospitals themselves to be contaminated due to some break in technique. Stringent additional controls, however, quickly reduced these losses.

It was also later found that certain flasks of plasma, produced at the beginning of the work and shipped abroad after tests had indicated that it was safe for use, had developed late bacterial growth. The Board of Medical Control on obtaining this information at once instituted vigorous and more extensive tests, including an independent check of all plasma by a central laboratory under the direct control of the Association. The English doctors were also simultaneously requested to report at once any further difficulties.

Upon receipt of the reports that these few flasks had been found contaminated, the Association cabled to England suggesting that thirty-six additional flasks, which had been produced at the same time and in the same hospitals as the contaminated material, should be discarded unless tests made over there showed it to be satisfactory. No word has since been received as to whether it was necessary to discard such additional amount of plasma, and the above is the only information which has been received to date of any difficulty from contamination, although the Association requested that it be advised immediately of any further trouble of this nature.

Of the total plasma produced for shipment to England, therefore, less than 1/6th of 1% has, on the basis of all reports so far received, been found after arrival to be unsatisfactory for use.

These difficulties with plasma produced in the first few weeks were, it is felt, not more serious than might reasonably have been anticipated in a new, pioneering project of this kind; and it is believed that, with the experience already gained, contamination can be effectively controlled, particularly if the work is done by trained personnel employed on a full time basis, instead of by volunteers. It is also hoped that experiments now under way, more fully discussed in the medical report, may at an early date evolve a method of removal of any chance contaminants by filtration.

As a part of the efforts to establish techniques protecting sterility, the doctors have given much consideration to the question of use of a so-called "closed system" for taking the blood.

This means a technique by which the air of the room is not allowed to come into contact with the blood. The conclusion of the Board of Medical Control on this subject is that the "closed system" should be favored.

The doctors have also carefully considered the possibilities of protecting sterility by use of preservatives, and the medical report contains detailed discussion on this subject as well as other methods of preventing bacterial contamination.

#### PROBLEMS FROM CLOTTING AND FROM CLOUDINESS OF PLASMA

In order to prevent clotting of the plasma, an amount of saline solution equal to that of the plasma itself has been added in all of the shipments to England. Meanwhile, work is being done to seek a means of avoiding this necessity, and to this end methods of clarifying the plasma are now being studied. It is felt that this would be a great advantage in several ways, and that this work should be continued to completion.

Such further experimentation also, it is hoped, will simultaneously solve the problem of cloudy plasma which, though not injurious in itself, makes it indistinguishable in appearance from infected material, and which therefore makes it additionally important if possible to develop a process which will produce clarity.

#### METHODS OF SEPARATING PLASMA FROM THE RED BLOOD CELLS

Methods of extracting plasma by settling and by centrifuging were thoroughly tested.

The work has shown that, by careful technique, sterility can be preserved in the centrifuging process, and that it is a more efficient method as to quantity of plasma realized, since it approaches the theoretical total yield possible, whereas the settling method was found to yield on an average about 20% less. Some type of centrifugation is therefore the method which our medical advisers recommend be employed for future use.

This may be done by the cup centrifuge now in use. But it is hoped that a more efficient quantity production method may shortly be evolved by employing a modification of the cream separator principle, which would permit a continuous flow. It is known that this has been used in recent months in England, but our doctors

are not informed as to how satisfactory it has proved. Such a machine has been ordered by the Association, and the possibilities of this method should be tested at the earliest possible date.

Large numbers of the cup-type centrifuges are available in hospitals and medical schools and could, it is thought, be borrowed for purposes of national defense. The setting up of a battery of such centrifuges would make possible immediate large scale production of plasma. Centrifuge bottles for these machines are also a stock item and immediately available in large quantities.

Much larger cup-type centrifuges could be specially built and would be more efficient than the smaller machines but would not be necessary if a successful adaptation of the cream separator principle could be made.

If speedy production is desired, a two-fold program is, therefore, recommended, including use of the smaller cup centrifuges for immediate purposes, and prompt testing of the possibilities of the separator.

#### WHAT IS THE BEST BLOOD SUBSTITUTE?

Throughout the work the attention of the doctors has been directed constantly to the fundamental question as to whether plasma is after all the ideal blood substitute. It is known that England now favors serum, apparently principally because sterility is more easily maintained in its production.

Stated in laymen's language, the difference between plasma and serum is that the former contains a portion of the blood essential to clotting which is not present in serum, as, in the production of serum, this is removed as a part of the coagulated red blood cells. Accordingly, in plasma, there is a tendency to formation of clots which present various difficulties.

Yet it seems to be generally conceded by all students in this field that liquid plasma properly prepared is innocuous in that it does not tend to produce serious reactions when administered, whereas there has been conflict in the medical reports concerning reactions following the use of serum. Experimental work which the Association has already caused to be started will, it is hoped, in a period of a few months give an authoritative answer to this question.

The doctors advise that, where it is proposed to store liquid plasma for long periods, deterioration is markedly diminished by adding an equal quantity of saline. This has the known disadvan-

tage of double bulk, and of perhaps slightly diminished therapeutic value due to the dilution, but it is known that, if properly produced, it will be an effective therapeutic agent.

The research sponsored by the Association includes work now in progress to attempt to eliminate the above noted objections to liquid plasma. The specific objective is a means by which to produce from plasma a clear fluid, which would have most of the physical advantages of serum and would also have the innocuous quality of plasma. If this can be done it is thought that it will be possible as a final step to put the pooled plasma through a very fine bacterial filter to assure sterility.

At the present time, at the request of the British Red Cross, the Association's research workers are engaged in testing certain filters made in America which it is hoped may help to solve some of these problems.

While no prediction can be made, there seems good reason to believe that the difficulties in the way of such improvements may not be very great, and it is hoped that at an early date a successful method may be evolved. If this can be done, it will be an important step toward mass production of a blood substitute of proven value for national defense.

#### FURTHER RESEARCH AS TO THERAPEUTIC VALUES OF THE PRODUCTS IN LIQUID AND DRY FORM

Medical opinion on this subject is surprisingly conflicting and inadequate. Reliable information is urgently needed for any long range planning. Under the auspices of the Association, there was therefore commenced in December, 1940, an elaborate program of experiments, which is being carried on at three different centers, and which will thoroughly test the therapeutic values of serum and plasma, each in liquid and dry form. Details of this are set forth in the medical report. A small quantity of blood to begin these experiments has been obtained, but it is estimated that a total of 4,000 blood donations for this purpose may be needed. In proposing the program incorporated in its memorandum of December 30, 1940, to the American Red Cross, the Association therefore contemplated that a part of the blood obtained from the proposed program of 500 blood donations per week, which would be processed in a central laboratory to be set up, might be used for the purpose of such experimentation in therapeutic values.

It is believed that a most important result of such research would be a definite conclusion as to whether dried plasma and dried serum are innocuous. Satisfying answers to these questions would obviously be not only of great value, but apparently absolutely requisite, before any very large scale production of such products in a dried form could be undertaken with complete confidence.

#### EXPERIMENTS IN DRYING PLASMA

Work has also been done by certain members of the Board of Medical Control of the Association relative to processes for drying plasma, and a new type of apparatus less expensive to build than other machinery sometimes employed for this purpose has been developed at Memorial Hospital. This apparatus consists of small individual units. The cost of 100 of such units would not exceed about \$3,000,—and might be very substantially less—and would have a capacity of drying plasma from 500 blood donations per week. Further testing and use of this process is recommended as an integral part of the experimental program proposed, together with tests of other drying processes now being developed elsewhere.

#### IMPORTANCE OF A "PILOT" LABORATORY TO CARRY ON THE RESEARCH NOW IN PROGRESS AND PERHAPS AS AN ADDITIONAL PRODUCTION UNIT

In the memorandum submitted by the Association to the Red Cross dated December 30, 1940, it was contemplated that the program therein proposed of 500 donations per week for a three-month period, to be processed into plasma in a central laboratory, would serve to carry on the pending research on all of the above subjects. Knowledge derived from actual experience on some or all of them is believed to be essential to avoid waste, disappointment and perhaps more serious consequences in mass production efforts. If some of these test procedures prove successful, it would demonstrate that it is possible and practical to set up a small relatively inexpensive laboratory in each city, where a donor campaign is to be conducted, to process plasma in liquid form without the attendant risks of long distance transportation of whole blood. It is also possible that these experiments may prove it to be practical to have such local laboratories dry plasma relatively inexpensively.

The laboratory, the setting up of which we advocate, would be a test model for such a program in other cities, would make possible continuance of the various studies and experiments above referred to in several fields, and could produce both liquid plasma immediately, and also dried plasma at an early date if desired.

It is respectfully urged therefore that full cooperation, reasonable funds and a constant source of blood be provided to the Association in order that the work along these lines which is already in process may be carried on expeditiously to a satisfactory conclusion.

As an integral part of such experiment, simultaneous work should be carried on in the possibility of development of mobile units for collecting blood at the first-aid rooms of large stores, offices and plants, and perhaps many other places. In the work just completed we had various offers of blood donations from the staffs of large establishments and companies if this could be done in their first-aid rooms. Because of the risks of contamination, it was necessary to reject these offers, but the experience showed that the supply of blood could be greatly increased, the work of the campaign for donors facilitated, and the problem of appointments largely solved, if arrangements could be made to take the blood of donors at their places of work instead of requiring them to go in their limited leisure hours to a hospital or laboratory.

#### WASTEFULNESS DUE TO LOSS OF RED BLOOD CELLS

One serious question in any large scale blood plasma project is the inherent wastefulness through loss of the valuable red blood cells forming half of the content of the blood. In the work which has recently been done, a part of such red cells has been sent for experimental purposes to Dr. Karl Landsteiner of the Rockefeller Institute, who is also a member of the Board of Medical Control of the Association; some part has been used by the hospitals themselves, principally for cases of anemia and for research; and some part has been sent to City hospitals for similar use, all of this being done upon the basis that the red cells were a by-product which would otherwise be wasted.

If no better use can be found for the red cells, even this waste may of course be justifiable in order to obtain a blood substitute which can be preserved over long periods, which will be instantly ready for the emergencies of war or other catastrophes, and which

in certain types of cases may be more efficacious than whole blood itself. However, intensive further research efforts to determine possible useful employment of the enormous quantities of red blood cells resulting from such a program are called for.

#### **Administrative Problems Inherent in Extension of Such a Program to Other Cities**

To establish the necessary effective central control and direction in the several divisions of the work which such a program would call for, it is suggested that the following points deserve consideration:

(a) A central publicity bureau which would supply news releases, radio scripts, etc., to local publicity directors. Even while the program is confined to the New York Metropolitan area, it would seem desirable to have one bureau of publicity for this activity which would serve all the Red Cross Chapters of the Metropolitan area which participate.

(b) That the publicity program be pointed toward emphasis, not upon a request for single blood donations, but that the donors enroll as members of a panel to give blood at reasonable times as necessary for the national defense program, so that it would be understood by the donors that they would remain as a continuing unit in the work. Reports from England indicate that they have been successful there in building up a large panel of such donors from whom repeated blood donations are obtained, and it seems probable that this would furnish the best method of obtaining large quantities of blood.

(c) That the Red Cross itself run the appointment office or bureau which, in the project just closing, has been conducted by the Association. It would seem that this is a normal function of the Red Cross.

(d) That a thorough trial be made of mobile units to go to plants, large stores and offices to collect blood in first-aid rooms, as previously discussed, provided means are found of avoiding increased risk of contamination from taking the blood in a place other than a hospital or central laboratory.

(e) That one or more central stations for taking blood, which would be conveniently located for large numbers of donors, be

set up. While, if possible, such station should be at the same location as the laboratory which would process the blood plasma, there seems reason to believe that, if the blood is carefully transported very promptly after it is drawn, a short haul to a separate laboratory in the same city would not cause serious damage. If it is found that this can be done, it would do much to facilitate obtaining the blood to have a bleeding laboratory or station established in the Grand Central area, and another in downtown Brooklyn, which would be more convenient for donors in both Boroughs. The necessity of a long trek to hospitals far uptown or over by the East River has, as experience shows, been a great handicap in procuring donations.

(f) A corporate entity should be formed to conduct the rather large scale business which such an extensive bleeding program entails and which involves a responsibility for medical and technical procedures which the Red Cross has indicated it does not wish to take. In the program in New York, this function has, of course, been discharged by the Association, which assumed responsibility for the bleeding, processing and all technical procedures, and generally conducted the business of producing the plasma. The Association is, of course, willing to continue this work in any new program in New York if this is desired. But, for the extension of such a program to other cities, some corporate legal entity should be created which will discharge this function of assuming legal responsibility for the conduct of the entire business, including engaging and paying personnel, renting laboratories and offices, complying with the regulations of local Boards of Health, as well as with the State and Federal requirements for such work, and of being the legal entity liable for any failure of compliance, and for any claims for negligence, malfeasance, etc. by donors, or by persons who may later claim to have been injured by administration of the product produced; and which would also take out appropriate liability and other insurance, and constitute a responsible body to be entrusted with funds and to render proper account thereof.

For the reasons explained, it is not recommended that even the partial responsibility for this work which the various hospitals have assumed in the program just closing should be continued. And even if this should be done, there is still need for a centralized responsibility and supervision of the work such as the Association has furnished in the blood for Britain project. Furthermore, in

some other cities the hospitals are unlikely to be as well equipped with technical knowledge on the part of their staffs, with blood banks, and with other facilities, as have been the leading hospitals of New York which have shouldered the burden of the work just completed.

Unless therefore the Red Cross makes a fundamental change of policy, and itself assumes responsibility for taking the blood, and processing it, some responsible legal entity or agency should be created to assume, for programs in other cities at least, the function of taking the blood and preparing it for shipment. Also, if it should be found impractical to transport the whole blood for long distances, such agency would have to take responsibility for processing it into plasma.

It would seem very doubtful whether a local committee of doctors could fairly be asked, or would be willing, to assume such heavy business and legal responsibilities, duties and risks. But such a corporation could be guided and directed as to all medical and technical matters by a central or headquarters Medical Advisory Board, and this could if desired be supplemented by a local Medical Board in each city where a campaign is conducted, composed of leading doctors of the community. The presence of such local doctors it is thought would also tend to enhance the confidence of donors. Such local medical assistance would undoubtedly be obtainable upon a volunteer basis, but it is believed that it would hardly take the place either of a corporation to do the business and to bear the legal responsibilities, or of a paid staff, which had had previous training in such a project, to set up the laboratory, observe the necessary techniques and precautions in taking the blood, and, if the plasma is to be locally processed, to do that also; or of direction of the entire enterprise from headquarters. Such a group of local doctors could probably therefore be a capable adjunct of such a program, but could hardly be expected to run such a business enterprise involving all the administrative and legal responsibilities, particularly when it is remembered that as soon as possible a completely standardized procedure should be developed which would be identical in all cities.

#### THREE MAIN DIVISIONS OF THE WORK IN ANY SUCH PROGRAM

For the reasons stated, it is believed that the work on any large scale program divides itself naturally into the following parts:

(1) Obtaining, enrolling and making appointments for donors, which is the natural field of the Red Cross.

(2) The medical and technical side of taking the blood and preparing the plasma, or taking the blood for shipment.

(3) The business and administrative functions incident to (2) above, which could best be discharged by a corporate body, which would assume also full legal responsibility for the medical and technical procedures.

#### Position of the Association

The Association's purpose and basic attitude throughout has been to utilize the unique opportunities of the project just closing to the full to gain knowledge which would be made available without reservation for the use of the Red Cross, the National Research Council, and the Army and Navy, as is now being done by this report and the accompanying medical report. Neither the Association as such, nor individuals connected with it, have any corporate or personal ambitions to serve, and the sole desire of the Association, its officers and medical advisers, is to see that the work done serves the broadest usefulness.

#### Conclusion

We wish to thank the American Red Cross for permitting us to join with it as a partner in the activity just closing, and for the opportunity for service which this Association has thereby enjoyed.

Respectfully submitted,

BLOOD TRANSFUSION ASSOCIATION,

By JOHN F. BUSH,  
*President,*

TRACY S. VOORHEES,  
*Chairman of the  
Blood Plasma Division.*

January 31, 1941.



## PART II

### MEDICAL REPORT

submitted in behalf of the Board of Medical Control by the Medical Supervisor of the Blood Plasma Division, the Chairman of the Board, the Chairman of the Blood Plasma Committee, and the Assistant to the Board, Blood Plasma Division

#### I

#### Introduction and General Statement

##### *Origin of Project*

On January 26, 1940, the Board of Trustees of the Blood Transfusion Association, on a recommendation of the Board of Medical Control, voted to make funds available to Dr. John Scudder of the Presbyterian Hospital for the purpose of carrying out studies of a laboratory and clinical type, related to the preservation of and the use of plasma as a blood substitute. This piece of research was to be carried on for a period of six months at which time a preliminary report was to be given. Dr. Charles R. Drew and Dr. Kingsley Bishop were associated with Dr. Scudder in this research.

Elliott, Tatum and Nessel (Mil. Surg. 85:481, December, 1939) had already reported a technique for the preparation of plasma and recommended it as a substitute for whole blood adaptable for use during World War conditions. This was not the first time that plasma had been suggested for such use but it definitely marked the beginning of the present swing to its widespread consideration as a blood substitute.

Strumia, Wagner and Monaghan (Ann. Surg. 111:623, April, 1940) reported on the intravenous use of serum and plasma. This report had grown out of work dating from about 1927. To a large degree the conclusion reached by Dr. Strumia and his associates, that citrated blood plasma was a superior and safer blood substitute for intravenous use than serum separated after clotting, dominated the early consideration of this problem in relation to preparing large supplies for war purposes.

Strumia, Wagner and Monaghan (J. A. M. A. 114:1337, April, 1940) likewise reported that intravenous administration of

citrated blood plasma had proved to be an ideal means of restoring adequate blood circulation in patients suffering from secondary shock. These reports took on added significance for the Blood Transfusion Association since studies, financed by the Association and carried on for a period of over a year by Scudder, Drew, Corcoran and Bull (J. A. M. A. 112:2263, June, 1939) had shown rather conclusively that for all practical purposes whole blood could not be safely used when over two weeks old, and that its use on a large scale in war would be very greatly limited by the difficulties inherent in collecting it in large quantities in centers of civilian population and transporting it to armies in the field in time to be of real value.

Meanwhile, however, Levinson, Neuwelt and Necheles (J. A. M. A. 114:455, February, 1940) had reported on human serum as a blood substitute with results comparable to the results reported with plasma and likewise advocated the pooling of plasma or serum as a method of suppressing iso-agglutinins in order to increase the safety of such transfusions.

In England under the pressure of war, investigation was being carried on in this field and had advanced to the point that Edwards, Kay and Davie (Brit. Med. Jour. 1:377, 1940) had reported on the method of preparation and use of dried plasma for transfusions. Ward (Brit. Med. Jour. 1:301, 1918) had suggested the use of plasma there many years before but with the end of the first World War further work was not carried out.

In Canada, Best and Solandt (Brit. Med. Jour. 1:799, May, 1940) had likewise during the latter part of 1939 and early part of 1940 been carrying on extensive research both experimental and clinical, in an attempt to determine the therapeutic efficacy of concentrated serum as prepared by the Thalhimer method (Proc. Soc. Exp. Biol. 41:230, 1939).

#### *The Choice of a Blood Substitute*

On June 10, 1940, at the annual meeting of the American Human Serum Association, four papers relating to this problem were discussed:

1. The Use of Citrated Plasma as a Substitute for Whole Blood, by Dr. Max Strumia.
2. Some Factors and Observations on the Preparation and Preservation of Diluted Plasma, by Dr. John Elliott.

3. Serum Transfusions in Humans, by Dr. Sidney O. Levinson.

4. Absorption of Isoagglutinins from Human Serum, by Dr. William Thalhimer.

At the conclusion of these papers a round-table discussion was carried out concerning blood substitutes and the discussions at this time played a large role in the early choice of diluted plasma as the safest blood substitute for emergency use.

A summation of the discussion revealed several things:

1. That there had been few or no reactions of any consequence in the hands of anyone using diluted plasma even though it was admitted there were certain difficulties in its preparation when compared with serum.
2. That opinion was divided on the effectiveness and toxicity of dried plasma.
3. That while in some hands serum had been given in relatively large quantities without reactions, in the hands of others very severe reactions had ensued and that only one individual in the discussion had had an opportunity to use both under similar conditions and his feeling was that plasma was markedly superior to serum.

This opinion, while far from being entirely unanimous, no doubt dominated the feeling of this group and to some degree challenged any organization beginning the collection of blood substitutes for emergency uses to show good cause for using any other substance than diluted plasma, without leaving itself open to the charge that warnings concerning the toxicity of serum had been neglected. However, this freezing of opinion at an early stage of the development of large scale use of blood substitutes was unfortunate and has caused the creation of two rather definite schools of thought.

#### *Proposed Organization*

Pursuant to the arrangements made with the American Red Cross, as set forth in Part I of this report, and following preliminary meetings a committee was appointed by Mr. Bush to prepare a memorandum on the requisites for the preparation of pooled plasma. This committee consisted of Dr. E. H. L. Corwin, Dr. Charles R. Drew and Dr. John Scudder. Such a memorandum

was presented on July 1, 1940 and in its recommendations were made relating to the equipment and personnel which the cooperating hospitals were to supply and the equipment and personnel which the Blood Transfusion Association would supply. Recommendations were made by the committee in some detail concerning personnel, requisite floor space, supplies, preparation of equipment, serology, bacteriology, supervision, routines for non-centrifuge technique for the preparation of pooled plasma, criteria for the selection and protection of voluntary donors.

#### *Relation to National Research Council*

At this meeting it was reported that the Surgeon General of the Army had taken up the matter of transfusions for war emergencies with the National Research Council and that the problem had been put into the hands of a special committee of which Dr. Walter B. Cannon, of Boston, was the Chairman, and Dr. Cyrus C. Sturgis, of Ann Arbor, was Chairman of the Sub-Committee on Blood Substitutes.

#### *Purpose*

This program from its inception had two purposes, first, immediate aid for war casualties, and, secondly, gathering all information which would be of value to the armed forces of the United States in case of a national emergency. With this second purpose in mind, throughout the course of the project very close contact was maintained with Dr. Sturgis. Each new change in technique, each proposed extension of the work was referred to him for approval or suggestions in order that the work being carried on might be integrated into the studies of the National Research Council.

While these plans were in progress, France fell, but the dramatic withdrawal of the English from Dunkerque raised the question of British needs and steps were immediately undertaken to ascertain whether or not shipments of plasma to England would be of any help, since informal advices had been received that most of the mobile units of the Blood Transfusion Service of the English Army had been lost in Flanders.

After some delay word was received that such aid would be very acceptable to the British Red Cross and plans were made to go ahead with the project.

#### *Medical Committees and Personnel Directing the Project*

The work throughout was under the general direction of the Board of Medical Control of the Association.

The Blood Plasma Committee of the Board, organized September 20, 1940, consisted of Dr. C. P. Rhoads, Chairman, Dr. Lester J. Unger and Dr. David C. Bull. Matters pertaining to the medical aspect of the project were referred to this Committee for a statement of policy and procedure.

The Research Projects Committee of the Board took under consideration all proposals for research. Its Chairman was Dr. Rufus E. Stetson, who was assisted by Dr. Ward J. MacNeal and Dr. Reuben Ottenberg.

Dr. John Scudder acted as Assistant to the Board of Medical Control and Dr. Charles R. Drew as Medical Supervisor of the project.

#### *Initial Steps Taken to Protect Sterility*

The regulations of the National Institute of Health of the United States Public Health Service applicable to the preparation of biological products were obtained, and before any plasma was shipped to England Dr. W. T. Harrison of the Institute came to New York for a conference with the Association on the subject, as later herein set forth.

On August 16th it was voted to comply with all of the rules and regulations of the Institute applicable to the sale of biologics, which are set forth in a pamphlet of the Public Health Service entitled "Regulations for the Sale of Viruses, Serums, Toxins and Analogous Products", and dated February 25, 1935.

It was also voted by the Board of Medical Control that the following additional routines should be established:

- (a) Culture the pooled plasma flask.
- (b) Culture one of the several containers filled from each pool of plasma.
- (c) Take a final sampling—3 out of every 100—in the receiving room at the refrigeration plant, before such batch is placed in the shipment room for the Red Cross.

This plan was later submitted to, and approved by, Dr. Harrison.

It is important to note that, in the development of the program, it became clear that even full compliance with the rules and regula-

tions of the Institute applicable to the preparation and sale of biologics by commercial houses was not adequate to insure the complete safety of plasma saline solution prepared on a large scale.

*Compliance with Laws and Regulations  
Applicable to Biological Products*

Promptly after the project was commenced, a study was instituted through counsel of the legal aspects of the work in order to make certain that the activities being carried on complied with Federal, State and City laws and ordinances for production of biologics. On September 25th a meeting was held which was attended by counsel for the Association, a legal representative of the City, Dr. W. T. Harrison, Chief of the Division of Biologics of the National Institute of Health, representing the Federal Government, Dr. A. B. Wadsworth, Director of Laboratories and Research of the New York State Department of Health and Dr. Ralph S. Muckenfuss, Director of the Bureau of Laboratories of the Department of Health of the City of New York. The following decisions relative to these matters were made:

(a) Relating to Federal Laws

Dr. Harrison ruled that because the plasma obtained from volunteer donors was for shipment to England and did not involve a sale of any kind, nor bring into play the rules pertaining to interstate commerce, the Division of Biologics Control had no jurisdiction over the matter and could not issue any license to the Association.

(b) Relating to State Laws

The position taken by Dr. Wadsworth was that the project as being conducted did not violate State Law.

(c) Relating to City Ordinances

The City Department of Health was seriously concerned about

(a) The unsupervised production of plasma in quantity in the City whether for sale or otherwise;

(b) The regulation of production and sale of biological products.

Dr. Muckenfuss felt however that the Association need only concern itself with the problems raised by Section 120 of the Sanitary Code, which provides that no serum or vaccine may be sold or given away except by a person holding a Federal license. Mr. Wolf felt that he could advise the Board of Health that the present efforts of the Association and the Red Cross did not come within the provisions of the Sanitary Code because there is no sale or gift of serum *in the City of New York*. Dr. Muckenfuss stated his willingness to advise the Board of Health that the work was in the nature of an experiment and therefore did not come within the provisions of the Sanitary Code.

A letter, prepared by counsel, was sent to Dr. John L. Rice, Commissioner of Health of the City of New York, explaining the set-up of the plasma project and the nature of its work with a request for a ruling as to whether the Association's activities in this field came within the scope of the Sanitary Code. At a subsequent date a commission was appointed by Dr. Rice and after a thorough investigation of the entire set-up they ruled that the Association's work was being adequately controlled, that it could not be considered as violating the Sanitary Code, therefore, that it might proceed with all speed.

*Duration of Project*

The first blood was drawn for Britain on the day that the first bombings of London took place, August 16, 1940.

As outlined in Part I, in November, when the project was reaching its greatest point of efficiency, word was received from England that the organization there had reached a point such that it could, early in 1941, supply the need for plasma. The work here was continued until January 17, 1941, the latter part of the program gradually shifting over to a consideration of the many problems which had arisen and the setting up of experiments designed to answer them, in order that such information might be available to the National Research Council.

Between August 16th and January 17th 14,556 bloods were drawn and about 5,500 liters of plasma saline solution were produced for shipment to England.

Though reports from England concerning the plasma have been meager, it is hoped that it has served a good purpose. It is certain that much has been learned, and many questions have been

raised which when answered will mark definite advances in the knowledge of blood plasma therapy. With the experimental program now under way as a result of this project, it is hoped that many of the mistakes made in this first large project of its kind may be averted in any future program and that the experience gained will be of service to this nation.

### *Disposition of the Plasma*

Of the total plasma produced, as above mentioned, 4,712 liters were delivered to the American Red Cross for shipment to England as soon as they passed the Association's tests, and have been, or are being, shipped to the British Red Cross in the first shipping space available. The balance is on hand pending completion of tests and will shortly be ready for delivery. It is known that at least one shipment, consisting of 222 liters, was lost by the sinking of S. S. Western Prince. Losses from other causes are dealt with in a subsequent section of this report.

The reports from England as to the use of the plasma have been too inadequate to enable us to make any comprehensive comment at this time.

## II

### Routines

#### (1) RECEPTION AND CARE OF DONORS

Throughout the course of the project every care was taken to reduce to a minimum the fears and discomforts of the volunteer donors. Many of the donors entered the hospital for the first time. The vast majority had never given blood for a transfusion. Many came with a spirit of almost determined martyrdom, not knowing what was expected of them, nor knowing what to expect from the persons they met. In the hospitals it was found useful to establish a routine of rather specialized service by many individuals in order to treat each donor as an individual worthy of every consideration, and at the same time introduce methods which resemble in some degree a factory line. A donor presenting himself at the hospital went through the following procedure:

#### 1. *Reception by a Red Cross Nurse.*

This nurse was usually stationed near the entrance to the building, was dressed in uniform and was chosen largely because of her ability as a receptionist. It was she who greeted the donor and escorted him through the maze of strange halls and by-ways of the hospital.

#### 2. *The Recording Secretary.*

She received the letter of appointment from the donor and checked off the name against the list of names which had been sent out from the Central Office for that day. Into a standardized record book such information as the name, age, address, etc. was entered and the hospital record card was filled out at the same time. This card was given to the donor to carry with him through the next series of steps.

#### 3. *Determination of Hemoglobin of the Blood.*

The Board of Medical Control felt that no donor with a hemoglobin of less than 80% should be used. This step ruled out quite a number of would-be donors. Persons found to have a marked polycythemia were taken in the early part of this project, but later rejected because the amount of plasma obtained did not warrant the expenditure of time and effort required to collect the blood.

#### 4. *Determination of the Blood Group by a Technician.*

This was done for three reasons: first, for statistical purposes; second, for the purpose of pooling the blood; and third, in order that the donor might have his blood group printed on his Red Cross Card of Thanks.

#### 5. *Taking of Temperature by Nurse.*

Earlier experimental work done under the auspices of the Blood Transfusion Association had demonstrated that the blood from any person running a temperature over 99.6 would almost invariably cause a reaction in the recipient. Hence the rule was made for this project that no person with a temperature of over 99 would be acceptable.

#### 6. *Determination of Blood Pressure by Physician.*

No donor was taken with a systolic pressure less than 110 mm. of mercury. It was felt that hypertension was not a contraindication for phlebotomy; hence, there was established no upper limit for blood pressures.

#### 7. *Physical Examination by Physician.*

This consisted of a history of general health and background as well as specific information concerning malaria, tuberculosis, heart disease and syphilis. History of any one of these four was immediate cause for rejection. The physical examination consisted of a thorough examination of the mouth, throat, heart, lungs and skin. The question was raised as to the necessity of examination of the genitalia, and the opinion of the Board of Health of the City of New York was obtained to the effect that in such a project examination of the genitalia might be omitted. The basis for this judgment lies in the fact that specific tests were done on each donor's blood for syphilis which would rule out all but the very earliest infected cases. Likewise, in stored blood spirochaetes cannot live over five days, so that in the case of reception of a blood from a recently infected case there is in fact little danger of transmission of the disease, since the processing of the plasma required at least three weeks.

#### 8. *Preparation of Donor's Arm by First Nurse Assistant.*

For the protection of the donor's clothes usually the outer garments were removed and a white hospital gown provided. The arm of choice was laid entirely bare and painted with iodine from the shoulder to the wrist. The iodine was then removed with 70% alcohol. The hand was wrapped in a sterile towel and a sterile towel draped to cover the entire arm except the region of the cubital fossa.

#### 9. *Phlebotomy by Physician.*

All doctors and nurses in the phlebotomy teams (in contradistinction to those doing the physical examinations) were completely garbed as for a surgical operation in sterile gowns, caps, masks and gloves. An amount of blood not exceeding 500 cc. was drawn from each donor into a bottle containing 50 cc. of 5% citrate solution. No "cutting down" on the vein was ever permitted.

10. When the phlebotomy had been completed, the second nurse assistant bandaged the donor's arm with a small neat bandage and sat with the donor for about ten minutes to be sure that there were no after effects.

11. Labels were put on the bottles containing the blood, and the test tube containing the sample for serology test by a secretary or volunteer, in the presence of the donor in order that there might be no mistake in identification.

#### 12. *Refreshments.*

Donors were supplied at each hospital with refreshments. These consisted of coffee, milk, or tea, with sandwiches or crackers and through the kindness of some of the larger distillers spirits were available to those who desired them. After refreshments, a physician, usually the chief of the clinic, after determining that the donor was in good shape, discharged him with thanks.

#### 13. *Accidents.*

In the entire project there were no serious accidents. There were no infected arms as a result of the blood giving. There were four or five cases of syncope in which minor wounds were sustained. These were cared for by the medical staff of the hospital at which the donor fainted. There were a few hematomas which gave trouble. Everything possible was done to assure the donors that this type of lesion, while bothersome for a few days, was not dangerous. The chief of each depot made it his personal job to follow up these cases.

### (2) COLLECTION OF BLOOD AND PREPARATION OF PLASMA

At the beginning of this project it was decided that each of the cooperating hospitals would use what equipment it had available to begin the collection of blood with as little delay as possible. Various methods were used to siphon the plasma from the sedimented blood into pooling flasks of various types, before final dispensation into uniform containers supplied through the Central Office. This resulted in a variation of techniques.

Two hospitals throughout the time of the project used the centrifuge system where the taking of the blood was done in a relatively open method with surgical care. One hospital began



using both sedimentation and centrifugation, later gave up centrifugation. Five began by using dumb-bell shaped sedimentation bottles fitted with a double hole para-gum rubber stopper, containing one piece of glass tubing to which a suction pump was attached, while the other glass tubing was attached to the phlebotomy tubing and needle. In two hospitals this first stopper was allowed to remain in the bottle and the rubber tubing was pinched off to maintain a closed system. In three hospitals the two hole stopper was removed under sterile conditions and a bakelite cap or solid rubber cork was inserted to remain there until the time for pooling.

Attempts were made soon after the beginning of the project to make more uniform the method of collection. The Board of Medical Control voted that all systems of collection should be closed but that hospitals maintain the right to obtain the plasma by centrifugation or by sedimentation. The results varied to a great degree with the experience of the individual operators regardless of the type of system used. Following the collection of blood the steps were about as follows:

1. Immediate refrigeration at a temperature of 4° C.
2. A diagnostic test for syphilis was done on the sample collected for that purpose.
3. In those instances where centrifugation was used, the blood was spun down at 2,000 revolutions per minute for one-half hour. Where sedimentation was used, the blood was allowed to sediment in the early days until a negative serology report had been received. Later it was decided to let the blood stand for at least a week in order that a greater amount of plasma might be obtained.
4. The plasma from an average of 8 bottles of blood was siphoned off into the pooling flask for the purpose of suppressing the agglutinins. This process was carried out in dust-proof, in most instances, air-conditioned, and preferably ultra violet lighted rooms, under a hood. For suction, either a pump of the Hyvac type or a water pump was used. When a mechanical pump was used there was interposed between the pump and the pooling bottle a water-trap in order to prevent any reflux of air into the pooling bottle. At the point where the trap was inserted into the system a break was provided in the form of a connecting tube filled with cotton so that at the time of taking down the apparatus any air

entering the bottle would be filtered. Between the pooling bottle and the donor bottle was interposed a three-way sterile stop-cock which served three purposes: 1) regulation of the amount of vacuum; 2) regulation of the flow rate of the plasma; 3) provision of a mechanism for extruding any cells accidentally picked up from the donor bottle. Behind this stop-cock there was interposed a stainless steel 120 mesh filter to prevent any larger clots of blood or fibrin from entering the pooling bottle.

5. At this point a sample was removed from each pool for aerobic and anaerobic cultures carried out according to the instructions outlined under Bacteriology.

6. Merthiolate was added in a quantity sufficient to guarantee dilution of 1:10,000 in the final plasma saline mixture. Merthiolate powder was added to sterile distilled water buffered by sodium borate and autoclaved. Sodium borate was added in the ratio of 1.4 grams to 1 gram of Merthiolate per 100 cc. of distilled water. Such a solution was used at the rate of 20 cc. of the freshly prepared 1% solution for each 1,000 cc. of plasma.

7. Such pools were then set aside in the refrigerator until negative cultures had been reported at the end of one week's incubation.

8. Final dispensing was carried out in an aseptic manner similar to that under which the pooling had been done. 500 cc. of the sterile plasma was added to each Plasmavac, a Baxter bottle which contains 500 cc. of sterile pyrogen-free physiological saline under 13" of water vacuum.

In this project the plasma was not filtered through bacterial filters but it is strongly recommended that in any future project efforts be made to make possible such filtration before final dispensing.

9. At the time of final dispensation samples of the Merthiolated pools were drawn off into small tubes containing physiological saline from the same batches used to fill the Plasmavacs. These pilot tubes were sent to the Central Laboratory, under the direction of Dr. Frank L. Meleney and supervised by Miss Balbina Johnson, for a final bacteriological check. This step was instituted for two reasons:

- (a) To check the bacteriology of the cooperating hospitals and,
- (b) to check the quality of the saline and the preparation of the equipment used for the final dispensing.

10. These 1,000 cc. Plasmavacs were packed in cartons of 6, sealed and stamped in such a manner that any entrance into the cartons would be immediately suspected. These cartons were held in a central refrigerated store-room until final shipment, when they were crated in groups of 6 or more in wooden boxes.

11. In England samples from each carton were tested bacteriologically before release to emergency stations for use.

12. Throughout the process accurate records were kept by serially numbering each donor flask, each pooling flask, each final container and each carton. Each flask or container in the series carried also the numbers showing the sources of its contents.

### *Summary*

1. The closed method of collecting blood is superior to the open method.

2. Centrifugation is preferable to sedimentation for the collection of plasma.

3. All bloods should be tested for syphilis.

4. The group of each donor should be known.

5. Plasma from each pool should be cultured before final dispensing. Samples of the final product should be cultured. In any system which does not provide for bacterial filtration, it would likewise seem advisable to culture the plasma from each individual blood before pooling is carried out.

6. A water-trap filter system should be instituted in any process of removing plasma by the siphon method.

7. In this project the plasma was not filtered through bacterial filters because of technical difficulties. It is hoped that, with the experiments now being carried out with various types of filters for the purpose of working out these details, this very essential step will be made possible for all future projects.

8. The routines followed in culturing serums as established by the regulations of the United States Public Health Service seem inadequate to insure sterility of plasma.

9. Of the total number of persons sent to the hospitals, about 5% were rejected because of history of communicable or organic disease, anemia, low blood pressure or general debility.

### (3) SEROLOGY

*Tests for Syphilis:* In accordance with the regulations of the Department of Health of New York City, the following routines were established for the testing of the bloods of the donors for syphilis, for reporting the findings to the Department of Health and for the follow-up concerned with the after-care of donors whose blood gave evidence of the presence of syphilis. The following instructions were sent to the hospitals:

1. The Kline, Eagle, Kahn or Hinton tests may be used as the rapid, first or presumptive test for syphilis, depending entirely on the routine in the hospital concerned.

2. Any blood showing a definite positive reaction to any of these tests is immediately discarded. Under this rule, it is recognized that some good bloods will be lost. Samples of all such bloods are kept and tested by the Wassermann method. This clears the medico-legal-social aspect.

3. Bloods which have presumptive tests which are dubious should be set aside until the Wassermann report is had. If in the opinion of the individual bacteriologist a one plus Kline is a dubious finding, such a blood may be set aside until the Wassermann is reported, but in no case is such a blood to be used until there is confirmation of its innocuousness.

4. A report of all positive tests for syphilis is to be made to the Department of Health of the City of New York. This is the law. We must abide by it.

5. It is suggested that the hospital authorities get in touch with each person who has had a positive report to discuss the matter with him in a manner similar to that which would be used with any private patient. The Board feels very strongly that this is an obligation that the hospitals should fulfill. A record of each rejection is kept in the Central Office but no record of the cause of rejection. This is a safeguard against information concerning serology getting into the wrong hands.

Because statistics from local blood banks had shown that the incidence of syphilis was very low in donors, throughout this project bloods were taken for the tests at the time of the bleeding rather than before. This was done to save the donors an extra trip to the hospital.

There were 14,556 individual bleedings: of these 151 were rejected because of serological evidence of syphilis: this represents a rejection of 1.03%.

#### (4) BACTERIOLOGY

At an early date it became obvious that the more important steps in the processing of the plasma would have to be carried out by a relatively few well trained individuals and that greater precautions would have to be taken to insure sterility than those outlined in the Federal rules and regulations which were followed at the beginning of the program. This conclusion was forced upon the Association when it was found that pools of plasma, which had previously been found sterile on culture, revealed the growth of bacteria on later examination. It seemed therefore that, in addition to the bacteriological control exercised by the individual hospitals, there should be a central laboratory to check the work of each of the hospitals. To this end, Dr. Frank L. Meleney, Director of the Surgical Bacteriology Laboratory at the Presbyterian Hospital, was asked to act as a consultant to the Association in this very essential phase of the work. At the request of the Medical Supervisor he agreed to meet with a group of bacteriologists to work out a program designed to control the process more adequately and insure the safety of the final product. A meeting was thereupon held on September 30th at which the bacteriological aspects of the program were thoroughly considered. To this meeting were invited the hospital bacteriologists working on the project and representatives of the City Department of Health and of Eli Lilly & Company.

After long and careful consideration of the many problems involved, the conclusions, as to the use of a preservative and as to bacteriological routines which are hereinafter set forth, were reached:

##### *Preservative*

At the beginning of the project "Merthiolate" (Sodium Ethyl Mercuri Thiosalicylate, Lilly) had been selected as the preservative to be used. Growth of organisms in the presence of Merthiolate, however, again raised this question for reconsideration. To assist in this discussion Mr. Jamieson, one of the authors of the original

publication concerning the substance was invited to discuss with the bacteriologists the following points:

1. The optimum dilution of Merthiolate
2. Effect of autoclaving
3. Toxicity

Mr. Jamieson discussed the questions in the following manner:

"We have worked with Merthiolate and used it as the antiseptic of choice in biological work for about ten years. We feel that it is the best substance for the use to which you are putting it. The optimum dilution is 1:10,000. In dilutions of 1:1,000,000 it is bacteriostatic. A concentration of 1:100 is not detrimental to red blood cells. The effect varies of course with the type of organism and it is not as effective against spore bearing organisms as it is against the non spore bearing type. This is true of all antiseptics. There is nothing a 1:5,000 will do which a 1:10,000 dilution will not do. We feel that a 1:20,000 dilution is not sufficient for safety.

"Autoclaving will not destroy the properties of Merthiolate, but I would suggest that the Merthiolate be added to the plasma at the time of pooling and after primary cultures have been taken rather than to the citrate at the time the blood is taken. The addition at this time complicates the testing of the plasma for sterility.

"In relation to the third point, toxicity, we have introduced quantities of 50 cc. of 1% Merthiolate intravenously into human beings with no effect and have given as much as 180 cc. of the 1% solution intravenously in a period of five days with no ill effects. The solution contains 49% mercury. I agree with Dr. Meleney that in order to have cultures of any value, once the Merthiolate has been added, the plasma saline mixture would have to be neutralized and Dr. Meleney's suggestion that 1 cc. of 1% ammonium sulfide be added to each 10 cc. of plasma and 50 cc. of the media, I believe is a good one. The ammonium sulfide will precipitate mercury and render it inert. We have seen no toxic renal effects. All of our tests are done by means of bacteriological and biological procedures; testing the antiseptic against counted colonies of specific organisms and checking the findings by intra-peritoneal injections of the material being tested in laboratory animals.

"Merthiolate retains its power for about three years, and in this respect compares very favorably with phenol, which sometimes decreases its bactericidal power in as short a period as three months."

In the work of Smith, Czarnetsky and Mudd (Amer. Jour. Sci. 192:790, 1936) they reported the finding that plasma proteins rapidly inactivate 0.3 grams mercury as contained in Merthiolate, therefore, on purely theoretical grounds the amount of mercury in a 1:10,000 dilution of Merthiolate was probably inadequate.

This raised the question of the possibility of using a combination of Merthiolate in a dilution of 1:20,000 and 0.25% Phenol as reported in the work of Falk and Aplington (Amer. Jour. of Hyg. 24:285, 1936). The danger here it seemed would be the large quantities of phenol which would be given in large infusions of plasma so preserved for a 3,000 cc. transfusion of plasma preserved with 0.25% phenol would cause the introduction of  $7\frac{1}{2}$  grams of phenol intravenously. It was felt that, until further work could be done, it would be unwise to take chances on the introduction of such large quantities of phenol. Records of the introduction of such quantities of phenol were very scarce and inconclusive.

This group of consultants decided that for this project Merthiolate was probably the best preservative available and its use in 1:10,000 dilution should be continued.

#### *Establishment of Bacteriological Routines*

At the meeting above referred to on September 30th it was also decided:

1. That the bacteriology department in each hospital test each pool of plasma both aerobically and anaerobically before such material is put into the final containers for release from the hospital.
2. That a central laboratory be established at the Presbyterian Hospital under the directorship of Dr. Frank L. Meleney and under the supervision of Miss Albina Johnson to which a sample of every pool should be sent from each hospital for additional tests for sterility and toxicity.
3. That such samples be sent in small sterile vacuum bottles containing 25 cc. of saline, this saline to be from the same batches of saline used in the final containers for diluting the plasma. This suggestion was made by Dr. Unger and was proposed in order to obviate the risk of contamination inherent in entering the final containers to obtain samples for the final testing.
4. That all Merthiolate be autoclaved before use and added to each pool in a quantity sufficient to guarantee a 1:10,000

dilution in the final plasma saline mixture and should not be dispensed into the final containers until a negative culture had been reported at the end of one week's incubation.

With these conclusions the following instructions were sent to each of the cooperating hospitals:

#### DIRECTIONS FOR CULTURING PLASMA

##### I. ROUTINE TO BE FOLLOWED IN THE LABORATORIES OF THE COOPERATING HOSPITALS.

When plasma has been removed from 8-12 recipient bottles (depending upon the size of the pooling flask), 10 cc. of each pool is withdrawn into a sterile pipette for culture.

##### A. *Aerobic Cultures:*

5 cc. of the plasma from each pool is inoculated into an 8x1 inch tube containing 35 cc. of a modified Holman's 0.2% dextrose cooked meat medium. This medium is prepared as follows:

Stir 1 lb. of very fresh lean chopped beef heart into 1 liter of water (distilled preferred). This infusion should be left in refrigerator overnight.

Boil vigorously for 15 minutes. Strain through cheese cloth and restore to original volume with distilled water.

Add: Neopeptone (Difco)	1%	(10 grams)
NaCl	0.5%	( 5 grams)

and stir until dissolved.

##### For *Dextrose Meat Medium*

Adjust pH to 8.4.

Boil for 20 minutes, restore to original volume with distilled water, filter through paper until clear.

Add 0.2% dextrose.

The chopped cooked meat is washed in strainer under running water to remove fine particles, allowed to drain, and excess water squeezed out.

The cooked meat medium is to be distributed into tubes, 8" x 1", filled  $\frac{1}{4}$  full with cooked meat, and at least 35 cc. of broth added.

Tubes are autoclaved at 15 lbs. pressure for 30 minutes. Final pH should be 7.4 to 7.6.

### B. *Anaerobic Cultures:*

5 cc. of the plasma from the pool is incubated into an 8" x 1" tube containing 40 ccs. Brewer's dextrose Thio-glycolate medium (J.A.M.A. 115:598, August 24, 1940). This medium may be procured from the Baltimore Biological Laboratories in Baltimore, Md. It contains:

Pork Infusion solids (from 37.5 gms. pork) ..	1.0%
Peptone-Thio .....	1.0%
Dextrose .....	1.0%
NaCl .....	0.5%
Sodium Thioglycolate .....	0.1%
Agar .....	0.05%
Methylene Blue (1:500,000) .....	0.0002%

Dissolve 3.65 grams in 100 cc. of distilled water. Heat until the solution boils and allow to boil about one minute. Autoclave 20 minutes at 120° C., 40 cc. to each tube. Do not store the powder in the refrigerator because this decreases the duration of anaerobiosis.

Both tubes are to be inoculated at 37° C. for two weeks. Examination of standard preparations of these tubes are to be made after 3, 7 and 14 days. If bacteria are seen on stained preparation, transfusions of 0.5 cc. are to be made to fresh 0.2% dextrose cooked meat media. (Tubes 6" x 5/8"), and 0.2 cc. is to be transferred to each of two blood agar plates, one for aerobic incubation and the other for anaerobic incubation. The organism should then be identified.

If at the end of 72 hours all cultures from each pool are negative, then that pool may be dispensed into final containers. If the 7-day or 14-day examination shows evidence of contamination not found at 72 hours, this should be reported to Dr. Meleney's Laboratory at the Presbyterian Hospital. Otherwise it will be assumed that the pool was free from contamination.

If the 72-hour examination of the pool shows a contaminant in both tubes, the pool is immediately discarded. If there is a contaminant in only one of the two tubes, 20 ccs. of this pool should be recultured (10 cc. aerobically and 10 cc. anaerobically). If after one week these cultures are sterile, the pool may be bottled.

### II. DISTRIBUTION OF POOL INTO FINAL BAXTER PLASMAVAC BOTTLES.

Immediately following the withdrawal of the sample of plasma from the fresh pool for culturing, there should be added to each pool sufficient buffered 1-100 Merthiolate to

form a dilution of 1:10,000 Merthiolate in the final plasma saline mixture. The safest way to be sure of the right proportion is to add 40 ccs. of a freshly prepared 1% solution to each pooling flask containing 2,000 ccs. of plasma. A simpler method is to add 10 ccs. of a 4% solution of Merthiolate to each 2,000 ccs. pooling flask.

It has been suggested that the Merthiolate powder be added to sterile distilled water and buffered by means of chemically pure sodium borate in the ratio of 1 gm. Merthiolate to 1.4 gm. sodium borate per 100 cc. distilled water. Or, in case of the stronger solution, 4 gm. Merthiolate to 5.6 gm. of sodium borate per 100 cc. distilled water.

Such pools are then set aside in the refrigerator until the 1 week culture reports are returned. If these reports are negative, then each pool may be dispensed into the final containers. Each Plasmavac contains 500 cc. physiological saline, to which is added 500 cc. of the pooled plasma. The last 35 cc. of plasma in the pool is run into a special control bottle containing 35 cc. of normal saline from the same batch of normal saline used in the Plasmavacs. This special bottle is to be labelled with the date, the name of the hospital, the number of the pool, and the carton number in which the pool is packed. These control bottles will be sent with the carton to the warehouse where a messenger will pick them up each day to carry them to the central laboratory, which is situated in the surgical bacteriological laboratory of the Presbyterian Hospital. Here rechecks of the pools will be done at 3-day, one-week, and two-week intervals, before the cartons are finally released for shipment.

### III. POSITIVE CULTURES IN CENTRAL LABORATORIES FOLLOWING NEGATIVE CULTURES IN HOSPITAL LABORATORIES.

Should a positive culture be obtained in the central laboratory on a plasma pool which has already been released by the hospital and sent to the store-room, the whole pool will be brought to the central laboratory and each flask in that pool will be separately cultured. The hospitals therefore should have readily available records of the serial numbers of the plasmavac bottles which were put up from any given pool.

The same procedure will be carried out if the two-week findings on the individual hospitals should be positive following a negative one week culture. This information is to be transferred to the central laboratory at once so that such pools may be retrieved from the storehouse and recultured.

#### IV. TOXICITY TEST.

In order to add a factor of safety and to come within the spirit of the law, one mouse will be injected intra-peritoneally with 1 cc. of plasma from each control bottle, and observed for 72 hours. It is not necessary for this toxicity test to be done by the individual hospitals.

#### *Central Bacteriological Laboratory*

The Central Bacteriological Laboratory for the project has been located in the Department of Surgery, Presbyterian Hospital. Dr. Frank L. Meleney, Director, released for the organization of the Central Laboratory and the supervision of the bacteriological studies the services of Miss Balbina A. Johnson, who is Assistant Director of the Laboratory. Full time services were given from October 10th to November 15th, and part time services have been continued to date. Dr. Sandusky has also contributed his services (about 2 hours daily) and assisted in the sampling of the pilots. The services of two assistants have been furnished by the Committee—those of Mrs. Maybelle Ivanoff from October 1st to date, and of Mrs. Marjorie Clark from November 15th to date. The preparation of media, washing of glassware, etc. was contributed by the Surgical Bacteriological Research Laboratory during the month of October. Salary for a part time worker was furnished by the Committee from November 1st to date. This work has been distributed between two regular workers in the Surgical Bacteriological Laboratory, Mrs. Boardman and Mrs. Razmouvsky. Under these conditions the necessary materials could be prepared in four hours working time daily. It could not be accomplished in four consecutive hours however.

The entire equipment of the Surgical Bacteriological Laboratory has been at the disposal of the Central Laboratory, i.e., dust proof room, incubators, sterilizing ovens, autoclave, microscopes, etc. Necessary supplies, such as media, glassware, syringes, mice, etc., have been supplied by the Committee.

The following is an outline of the procedures actually employed in the Central Bacteriological Laboratory which was prepared by Miss Balbina Johnson:

*Technique:* Tests on Pilots for Plasma Saline Mixture Containing Merthiolate 1:10,000. (This material represents plasma remaining in pool after the final containers have been filled.)

*Media:* 0.2% dextrose cooked meat medium for aerobic cultures. Brewer's thioglycolate medium contain dextrose and Eh indicator for anaerobic cultures.

These media are tubed in 8" x 1" tubes in 45 cc. amounts. All baskets of media are kept tightly covered until inoculated to prevent dust from collecting on cotton plugs.

*Sampling:* All test pilots are allowed to stand 1 week at room temperature before sampling is done.

The appearance of material in the test pilot is noted before sampling, i.e., undue cloudiness, amounts of sediment; fibrin, etc.

All sampling is done by a team of two in a dust-proof room exposed to ultra-violet radiation. Each member of the team wears a sterile gown, cap and mask as well as adequate protection from the ultra-violet radiation. The metal covers are removed from the "pilot" culture tubes and the ultra-violet radiation allowed to play on the rubber covers for one hour before the pilots are sampled. Just before sampling, tincture of iodine is applied to the rubber covers. The "pilot" is always shaken to assure an even distribution of any possible contaminants. A 15 to 20 cc. sample of the plasma mixture is then withdrawn. A sterile 20 cc. glass syringe is used fitted with a 19 gauge needle. Care is taken not to introduce air into the "pilot" as this may result in contamination of the plasma mixture. If the rubber stopper leaks while plasma is being withdrawn or if the vacuum has been lost, this is noted. Four tubes of media are used for testing each pilot, two—0.2 per cent dextrose meat medium, two—thioglycolate medium. One tube of each medium is inoculated with 5 cc. of material from the test pilot, the other with 0.5 cc. The needle is flamed to redness in a Bunsen flame before each inoculation. The material remaining in the syringe is introduced into a Wassermann tube for subsequent mouse inoculation. The cultures are incubated at 37 deg. C. for 14 days. All tubes are examined for visible contamination after 3 and 7 days of incubation at which time the cultures are shaken well. Care is taken to keep the culture medium from coming in contact with the cotton stopper. (Metal cap culture tubes are superior.) On the 14th day stained preparations (Gram) are made of all tubes. Sub-cultures (5 cc.) of all tubes showing suspicious turbidity are made even though no organisms have been seen on smear. The turbidity is at times misleading as the material in the test sample is often cloudy and may contain considerable sediment and shreds and flakes of fibrin, in spite of the dilution in saline.

All cultures showing organisms on smear are sub-cultured into liquid media and platings are made on 5 per cent sheep's blood agar plates for aerobic and anaerobic incuba-



tion. It proved impossible without increasing the laboratory staff to Gram stain all cultures on the 3rd and 7th day of incubation. However any tubes showing evidence of contamination at these examinations are immediately smeared and sub-cultured. All pilots with positive cultures are recultured and the Plasmavacs (final containers) from the corresponding pools are also sampled. The technic is the same as for the original tests on the "pilot" tubes.

Representative lots of discarded inoculated tubes in which no growth had developed have been inoculated with test stains as a check on the ability of the media plus the merthiolated plasma saline solution to support growth of organisms. In no instance have the organisms (staph. aureus hem. Strep., E. coli, Cl. welchii or B. subtilis) failed to multiply even though only a few organisms (1 to 10) were introduced. The effect of Merthiolate 1 to 100,000 or 1 to 1,000,000 in the cooked meat medium and in the theoglycolate medium has proven very different from its inhibitory effect in simple infusion or extract broth. Brewer (J.A.M.A. vol. 115, 1940, pg. 598) states that the sodium thioglycolate in this medium combines with and inactivates most of the mercurials used as preservatives. This medium also supports the growth of microaerophiles and aerobes as well as obligate anaerobes.

*Toxicity Tests:* 0.5 cc. of the plasma saline solution from each "pilot" is injected into a mouse. The mouse is kept for 1 week. If it dies, two additional mice are injected and if these survive and show no toxic symptoms the first death is disregarded.

#### REPORT OF CENTRAL LABORATORY AS TO PILOT BOTTLES CONTAINING MERTHIOLATED PLASMA SALINE SOLUTION

(Oct. 1, to Dec. 31, 1940)

Tested .....	836
Contaminated .....	16
No. of Strains Recovered.....	23

They were as follows:

<i>Staphylococci</i> .....	9
non-hem. Staph. aureus .....	8
non-hem. Staph. albus .....	1
Green Streptococci .....	4
Diphtheroids .....	4
E. coli .....	2
B. pyocyaneus .....	1

B. subtilis .....	2
Aerobic gram neg. sporulating bacillus .....	1
Cultures contaminated with 1 species.....	10
Cultures contaminated with 2 species.....	5
Cultures contaminated with 3 species.....	1

#### *Plasma Found in England to be Contaminated*

It was discovered in the Central Laboratory that certain pools of plasma, which had been examined after three days or one week and found to be free from bacteria, when examined later contained growth. This caused fear that a similar situation might be found concerning some of the early shipments to England. This proved to be the case. On November 14th a cable was received from England on this subject, and later more detailed information was received which disclosed that eight bottles had been tested and found to be contaminated. Immediate cable advices were sent to England to discard certain additional cartons containing 36 liters of plasma saline—which included all of the plasma from pools prepared on the same day at the hospitals from which the contaminated material was sent—unless such 36 liters of plasma were proved to be sterile by further tests to be made in England. This was done in order to be absolutely sure that no suspected plasma was used.

This information was transmitted to the members of the Blood Plasma Committee and to the Directors of the Plasma Banks in the hospitals from which the pools had come. A complete report was obtained from each of these hospitals, and it was striking to note that each of the bottles found contaminated in England had been tested aerobically and anaerobically before shipment and found negative for any growth.

The contaminated flasks were all prepared and shipped in August before the bacteriological technique was revised in accordance with the more rigid criteria, and before establishment of the Central Laboratory.

Further to safeguard future shipments, the following recommendations were made:

1. That pools be held at least one week instead of three days, and that negative cultures be reported before final dispensation into Baxter bottles.

2. That all suspicious material be sent back to the hospitals.

3. That plasma with positive pilots and negative pool cultures be discarded.

4. That any final container which has been entered be sacrificed.

There have been no further reports of contaminated plasma arriving in England.

### Summary

Experience in this project has shown that the collection, preparing and storing of plasma is a difficult task from the point of view of maintaining absolute sterility throughout. For any large scale program every step of the process should be checked by aerobic and anaerobic cultures at repeated intervals for a period of at least three weeks before the final product is released for intravenous use. The only safe procedure is to culture a sample of each individual blood, to culture one or more samples from each individual pool of plasma and to culture one or more samples of the final product, be it liquid or dried, from the individual pools. This entails a great deal of work and expense but in the opinion of this Association, is entirely justified if the final product is to be given to sick individuals with absolute safety. In addition to this rigid set of bacteriological standards there should be used some preservative in order to prevent the growth of any chance undetected contaminant. "Merthiolate" (Lilly) has been used in this project but it is felt that the ideal preservative has not been found.

The bacteriological reports are not complete but the estimated figures are about as follows:

Total amount of plasma saline produced, or put in process of production in hospitals .....	6,151	liters	
Amount found contaminated at hospitals	361	"	
Amount found contaminated in Central Laboratory .....	160	"	
Percentage contaminated .....	8.5%		*
Lost through breakage, clotting, etc....	60	liters	
Unreported but held pending special further tests .....	74	"	
Total lost through all causes .....	581	"	**
Percentage lost through all causes .....	9.4%		**

\* Does not include 8 liters found in England to be contaminated, or 36 liters which may have been contaminated.

\*\* Does not include that held pending further tests, or the small amount found contaminated in England.

### (5) UNIFORM RECORDS OF BLOOD AND PLASMA SHIPMENTS

Each hospital instituted at the beginning of the project its own bookkeeping methods in order to keep track of donors, the bloods, the pools, and the plasma shipments. This led to great confusion in attempting to summarize the records in the Central Office. In order to simplify and make more uniform the bookkeeping the following suggestions were made to the cooperating hospitals:

That all records be kept in a master book (pages 14 x 11 inches) supplied by the Blood Transfusion Association, which contained printed headings over spaces on the left-hand page for: date, serial number of the donor; name; sex; age; address; group; and the result of the test for syphilis. While on the opposing right-hand page the following columns: date of pool, pool flask number; culture; test tube from pool; final container number; the amount of plasma and sodium chloride in the final container; the result of the culture both aerobic and anaerobic on the final container; the pH of the final product; carton number; consignment date; reculture date from warehouse; and remarks. The following instructions were sent to each cooperating hospital:

In order to simplify and make more uniform the bookkeeping associated with the taking and shipping of plasma, the following scheme is suggested:

1. That all donors have a serial number and that this number appear on each donor bottle. In referring to it the abbreviation D 1-100 will quickly differentiate it from other containers.

2. That the pooling flasks likewise be numbered serially from 1-100, and be referred to as P1, P2, etc.

3. That all cartons be numbered serially beginning with No. 1. It will then be possible to record data briefly, e.g., P25 in C34.

4. That each Baxter Plasmavac be numbered serially. The record will then show P25 dispensed into B 106-112 inclusive.

When sending cultures to the central laboratory two numbers will be necessary, the pool number, e.g., P40, and the carton number, e.g., C28. When sending cultures to your local hospital laboratory only the pool number need be sent. If the pool is found to be contaminated it will be an easy job to find carton No. 28 for rechecking.

## III

## Equipment and Processing

## (1) CONTAINERS

*Donor Bottle*

The decision as to the ideal container for each of the three steps in the preparation of plasma for shipment has been one of the most difficult problems. It would seem that the simple matter of a bottle to hold blood would be of very little import; such however has not been the case. In the beginning of the program the bottle recommended for use was a dumb-bell type Pyrex bottle for sedimentation as recommended by Scudder, Bishop and Drew (J. A. M. A., 115,290, July 1940). The advantages of this particular type of container had been proved to be as follows:

1. As a result of the small interface area between the cells and the supernatant plasma the rate at which the cells deteriorate is definitely slower than in containers when the area is much greater. (Scudder, Drew, Corcoran and Bull, J. A. M. A., 112:2263, June, 1939.)

2. The percentage of plasma obtainable from such a bottle for a given period of sedimentation is greater than can be obtained from bottles of different shape.

3. When made to hold 550 cc., 500 cc. of blood and 50 cc. of citrate, and filled to the top, the denaturation of the protein in the plasma goes on at a slower pace as a result of the absence of or smallness of the interface between the plasma and the air. With these advantages in mind this bottle was accepted as the standard container when the project first opened. In mass production, however, with many volunteer workers doing the phlebotomies certain disadvantages soon became obvious. These were:

1. A rather small but definite and constant percentage of bloods were lost through clotting as the result of the inability to mix thoroughly the citrate and the blood during the bleeding.

2. As a result of the very high percentage of women donors whose blood counts run far below those of the average male, the line of demarcation between the plasma and the cells in a large percentage of cases fell not in the narrow constricted neck but rather in the wide bulbous lower part

of the bottle. This of course defeated the purpose for which the bottles were constructed.

3. Some cells in many cases settled on the slope of the upper bulb and at the time of removing or siphoning the plasma were often sucked into the pooling flask thereby causing a plasma less clear than desirable.

It was felt, therefore, that this bottle should be redesigned to eliminate the faults which had shown up in the large scale use, or that some more simple straight-sided bottle should be adapted for use as the donor bottle. Work is being done along both of these lines. The dumb-bell bottle has since been redesigned and the new model is being retested. A straight-sided bottle has been developed and is being tested. It is impossible at this time to give a final answer as to the efficacy of either as the bottle for collecting the blood.

As a stop-gap container the Haemovac bottle put out by the Hospital Liquids Supply Company, was used. Its chief virtue is the fact that it is relatively cheap. The Hospital Liquids Supply Company has been very generous in their attempts to create a bottle more suitable for this particular type of job and such investigation has been carried out with certain criteria in mind.

In the beginning of this investigation it was felt that a bottle should be designed which was capable of yielding a high percentage of plasma when sedimentation alone was the method of choice; or a bottle capable of being centrifuged where centrifuges were available. The difficulty at this step was that there was no stock bottle with a 600 cc. capacity and there is no centrifuge made with a head to hold such a bottle. To create such a bottle involved a cost of approximately \$1200 for the original mold and first order and a similar outlay for the creation of a centrifuge to hold such a container. Such expenditures it was felt were not justified at the time.

In some of the hospitals which cooperated in this work the blood has been centrifuged from the very beginning. The centrifuge used is the No. 2 International with a head containing 6 Trunnion Cups to hold bottles of 250 cc. capacity. Actually the centrifuge bottles which are a stock item have a capacity of only 230-240 cc. This means that for each 500 cc. phlebotomy at least two bottles would have to be used. This doubles the work, doubles the chances for infection, and increases expenses but yields a high percentage of plasma and *in an emergency could be set up on a large scale at once.*

To establish true differences in the yields of plasma using these various types of bottles, and comparing sedimentation with centrifugation as a method, measurements were made by Dr. John Scudder and Dr. Earl Taylor, at Presbyterian Hospital, Dr. Nathan Rosenthal, Mt. Sinai Hospital, and Dr. Lester J. Unger, of the Post Graduate Hospital. Their results were practically the same and in round figures were about as follows:

*Yield in Percentage of Plasma From  
Bottles of Different Types*

1. Dumb-bell bottle .....	43%
2. Haemovac bottle .....	37%
3. Straight-sided bottle $2\frac{3}{4}$ " wide with a wide bulb at the base.....	38%
4. Small Centrifuge bottles .....	53%

It is obvious that there is a marked saving of plasma when centrifugation is carried out. It is therefore desirable in any large program in order to obtain the maximum amount of plasma with our existing knowledge of processing to centrifuge all plasma. Should the plans now being worked out for the separation of plasma and cells by means of a modified cream separator prove successful, the bottle of choice would be the straight-sided bulbous-bottomed 600 cc. container.

*Pooling Bottle*

An attempt was made in the early days of the project to pool large quantities of plasma, from 5 to 8 liters, in order that there might be complete suppression of agglutinins but the danger of infection and contamination of such large quantities showed that it was wiser to use smaller pools. The standard pooling bottle used in this project has been the 2 liter Baxter bottle. This has not been ideal for our purpose. A 3 liter container would have been preferable, since the plasma in one pool would have been shipped in one carton, simplifying bookkeeping, identification and technical procedures in the hospitals. But the problem of obtaining such a 3 liter bottle required the expenditure of a considerable sum of money for the creation of molds and the ordering of a sufficient number to make it worth while for the manufacturer. Since there was no need for a great number of such bottles this 3 liter bottle has not been created. The 2 liter bottle however has proved workable if not entirely satisfactory.

*Final Container*

The first shipments of plasma saline to England were sent in two-liter bottles. Word was received from England that they would prefer to have a final container of one-liter capacity. The container adopted was that known as the Plasmavac put out by the American Hospital Supply Co. (Baxter). It has been supplied to us containing 500 cc. of pyrogen free, non toxic physiological saline and hermetically sealed with a vacuum of 26 inches of water. While the principle of high vacuum for the taking of blood has certain disadvantages it has worked extremely well in transferring plasma from the pools to the final container. The bottles made by the Baxter Company are not of the pyrex variety and will not stand up under boiling with alcohol or acids, which should be a routine procedure, whenever these bottles are to be re-used. Since every final container is used once and thrown away, it was felt that this was no particular handicap. A few of these final containers were smashed in transit and this raised the question of a more durable container.

*Plastic and Metal Containers*

To this end the Monsanto Chemical Company was consulted concerning the possibility of creating a container made of plastics. The results of this investigation showed that none of the plastics now on sale by any of the companies would withstand repeated autoclaving at the temperatures necessary to insure complete sterility.

Some of the Polystyrene plastics are transparent and may be chemically cleaned but will not withstand a temperature of 254° Fahrenheit. The Phenol-Phenolaldehyde are dark in color, therefore not entirely suitable as containers and further it has been shown that biological substances are partially absorbed by this material. Lucite, made by the DuPont Chemical Works can be obtained in transparent form and is very durable but will not stand repeated autoclaving. The failure to find suitable material in this class raised the question of metal containers, although it had been the opinion of the Medical Board that the final container should be one in which the condition of the plasma could be ascertained by simple examination since gross contaminations cause very marked changes in the appearance of the material. There are several tin containers on the market. One made by the Continental Can Company of

Philadelphia which can be silver lined and fitted with an air-tight cap would be suitable were it not for the question of visibility. Of perhaps greater practical importance is the fact that glass containers may be shipped in these tin containers under vacuum. This process would offer protection in two ways:—(1) a greater freedom from breakage and (2) less likelihood of absorption of air or moisture through the rubber cork in the glass container. Ideally of course the container or ampoule should be sealed by flame and no rubber stopper should be used since all of them have been shown to be permeable to air and moisture after a relatively short period of storage.

#### *The 800 cc. Bottle for Collection and Shipment of Blood*

It would seem unwise to establish a large round 800 cc. bottle as the standard for the collection of blood. The reasons for this opinion are as follows:

1. It is unwise to take over 500 cc. of blood from any single donor at one time. This means that there is an air-space of 300 cc. in which the blood can splash around while in transit from one place to another. If the plans call for shipping the blood from one city to another, the amount of damage done to the cells in such a container would be much greater than the damage done to the same cells in a container filled to the top.

2. If in order to obviate the physical trauma such a bottle is filled to the top with physiological saline or 5% glucose, then the protein content of the plasma saline mixture will be less than half its normal content and its clinical efficacy will be reduced in proportion.

3. The diameter of the large bottle is approximately 12 centimeters. Blood stored in such a bottle for over relatively short periods of time will show marked changes as measured by the rate of hemolysis, the increased potassium content and the decreased sodium content of the plasma as well as the more rapid changes in the fragility of the cells.

4. The use of such a bottle for centrifugation requires the construction of a custom-built centrifuge which costs in the neighborhood of \$1,400 to \$1,500.

#### *Summary:*

1. It is the opinion of this body that for any immediate program the most suitable container for taking blood is the 250 cc. stock centrifuge bottle or the 300 cc. Baxter Centrivac bottle, either of which will fit into the standard No. 2 International Centrifuge. Both the bottles and the centrifuges are immediately available.

2. More ideal would be a 600 cc. centrifuge bottle providing centrifuge heads suited to spin them could be created. Neither the bottle nor the centrifuge are at present available.

3. For the collection of blood for shipment and later separation by some means other than centrifugation the 600 cc. capacity bottle now being created by the Hospital Liquids Supply Company is recommended as an improvement over any of the bottles on the market at the present time.

4. For immediate purposes the 2 Liter Baxter Transfusovac is the most suitable container for the pooling of plasma. A 3 or 3½ liter container would be more ideal, but there is on the market at this time no such bottle with adequate capping equipment for the transference of plasma with a completely closed technique. If each sample of blood is cultured before pooling, pools of 10-50 bloods may be done with safety.

5. The 1,000 cc. plasma bottle as produced by the Baxter Company has proved suitable as the final container. When adequate methods of filtering the plasma have been worked out, it would seem no longer necessary to dilute the plasma with equal quantities of saline, the chief reason for this dilution being the prevention of fibrin precipitation on long standing. If this can be done, a 500 cc. bottle could be substituted as the final container.

6. Any set of bottles used should be provided with a stopper or capping device which will allow the collection of blood, the pooling of the plasma and the final dispensation in a closed system, i.e., without exposure to the air.

7. If shipment of the whole blood for any considerable distance is contemplated—a procedure which is not recommended—this should be done in a completely filled container, promptly after the blood has been drawn, and under constant refrigeration.

## (2) PLASMA PROCESSING ROOM

It soon became evident at the beginning of the project that it was not safe to siphon plasma and put it in its final containers in an exposed workroom or laboratory. To obviate this potential source of contamination each hospital was advised to have constructed an isolated, air-conditioned dust-free room or compartment for the exclusive use of the limited number of persons assigned to the job of pooling the plasma, taking cultures and dispensing the final product.

Such rooms may be of any size. The following specifications were suggested as a guide in constructing such compartments:

1. That a window be included in the wall plan for such a compartment in order that air from the outside may be brought in and exhaust air evacuated outside the building.

2. That, where such rooms were not already in existence, constructed of the usual tile and plaster walls, they be constructed of double wall composition boards and glass. The idea of the double-wall is to create a dead space between the inner and outer panes of glass in order to prevent the transference of heat or cold from or to the compartment.

3. That one of the standard air-conditioning machines equipped to filter all incoming air, to exhaust internal air to the outside when need be, to recirculate internal air, to humidify the air and to provide refrigeration capable of reducing the temperature in the compartment at least 10 degrees lower than the outside air should be used. Several standard pieces of equipment will answer these specifications, and for a room of modified size, let us say 6x8x10' to 10x14x10', a one-half horsepower unit with a capacity of about 6,000 B.T.U. per hour should suffice. The following units it is believed will answer this purpose well:

1. The Philco-York Air Conditioner—Model #61—motor size  $\frac{1}{2}$  size Horsepower—capacity 6,000 B.T.U. per hour.

2. Frigidaire Room Conditioner— $\frac{3}{4}$  Horsepower—dual voltage machine—using Freon-12 as the refrigerant.

3. General Electric Type F.B. 50— $\frac{1}{2}$  Horsepower or F.B. 70— $\frac{3}{4}$  Horsepower—the former having a net cooling capacity of

approximately 6,000 B.T.U. per hour, and the latter a net cooling capacity of approximately 8,000 B.T.U. per hour.

4. Carrier Room Air Conditioners—the small one  $\frac{1}{2}$  Horsepower unit with a capacity of 6,100 B.T.U. per hour—and the larger unit with a  $\frac{3}{4}$  horsepower—capacity of approximately 8,500 B.T.U.

5. Westinghouse Air Conditioning Units are obtainable in three sizes: the smallest— $\frac{1}{3}$  Horsepower “Mobilair” model W. A.-04 with a capacity of 4,000 B.T.U. per hour;  $\frac{1}{2}$  Horsepower unit with a capacity of approximately 6,000 B.T.U. per hour; and for a larger room of approximately 12x16x10'—Model F.A.-09— $\frac{3}{4}$  Horsepower “Mobilair” Unit with a capacity of 9,000 B.T.U. per hour.

These units range in price from \$150 to \$300 depending on the size and capacity. Each requires an air outlet to the exterior of the building in order to give the best results.

Should it be necessary to construct such a cooling unit in a room without window space it would seem advisable to use a water cooling unit such as the Westinghouse “Mobilair” Unit S.W.-06— $\frac{1}{2}$  Horsepower with a capacity of 6,000 B.T.U. per hour.

In addition there should be constructed a hood under which all of the processes should be carried out and as an additional safety feature it has been found advisable to have technicians garbed as for sterile operation with caps, masks, gowns and gloves.

In addition to lighting there should be outlets for gas, pressure, suction and additional electrical sockets. The burners used should be of the Micro-Bunsen type in order to cut down the heat production. If the suction apparatus is one of the Cenco Hyvac pumps, an altogether satisfactory unit, it should be placed outside of the plasma room with the suction lines led through the wall openings in the double wall.

The interior of the room should be painted with a water-proof covering in order that, from time to time, the whole room could be washed down in order to free the atmosphere from dust.

The installation of ultra-violet light units is an added precaution which seems well worth the rather moderate expense involved.

### (3) WORK ROOM

Probably the most important single step in cutting down the number of reactions from intravenous administration of nearly any type of material is the step which has to do with the cleansing of the equipment. To this end there should be a separate fully equipped work room presided over by someone who is fully cognizant of the disastrous effects of using improperly prepared equipment for blood or plasma transfusions. There should be adequate facilities for washing dirty bottles, needles and tubing, immediately after their use. Tanks should be provided with manifold outlets for the continuous washing of rubber tubing over long periods of time. There should be enough work table space to prepare the sets; there should be a small gas range for boiling small pieces of equipment; adequate autoclaving facilities; adequate source of pyrogen-free water and a large drying oven for drying glassware before autoclaving. Too much stress cannot be put on the importance of this phase of the work. Next to the technician who is directly responsible for the processing of the plasma, the person responsible for the preparation of the equipment is probably the most important cog in a smoothly running organization.

### (4) PLASMA DRYING UNIT

A description of the various types of apparatus which have been developed for this purpose—including the new and relatively inexpensive equipment recently designed at the Memorial Hospital—is omitted at this point, as it is given subsequently herein as part of the discussion under the heading Dried Plasma and Serum, Section V, *infra*.

### (5) PREPARATION OF EQUIPMENT FOR USE

All equipment used for the drawing of blood should be immediately rinsed with cold water before the blood has a chance to dry in the tubing and needles. Likewise all bottles in which blood has been stored should be rinsed thoroughly with cold tap water as soon as the blood has been removed from them.

All new glassware before use should be boiled for five minutes in a 0.1% solution of Sodium Hydroxide, rinsed thoroughly in cold tap water, rinsed with sterile pyrogen-free distilled water and dried at a temperature of 180° in an oven before autoclaving.

All old glassware between periods of use should be thoroughly washed in cold water, then in lukewarm water with either green soap, Duponal, or some other non-greasy cleansing agent, scrubbed, preferably by a rotor brush attached to a motor and arranged so that the continuous flow of soapy water can be played on the inside of the bottle as the brush is in action, thoroughly washed in tap water, boiled for five minutes in 0.1% Sodium Hydroxide, rinsed with tap water then at least five times with distilled water and dried at a temperature of 180°.

All new rubber tubing regardless of type should be prepared by preliminary soaking in diluted Sodium Hydroxide for 24 hours, individually washed with tap water and allowed to soak for 24 hours in tap water and finally rinsed with a continuous flow of distilled water for one-half hour. Black and red rubber tubing is likely to contain a larger percentage of sulphur in the rubber or on the rubber than the para-gum variety but even this contains sufficient to warrant preliminary preparation with Sodium Hydroxide.

The most important step in the preparation of rubber tubing for re-use is the immediate rinsing of the tubing with cold tap water until every trace of blood is removed as soon as the tubing has been used. It should then be boiled in 1/10% Sodium Hydroxide for three minutes, rinsed in tap water and finally rinsed in distilled water before autoclaving.

All needles should be rinsed as soon as used with cold water then thoroughly scrubbed with Bon Ami or some other non-corrosive cleansing material, then boiled for three minutes in Sodium Hydroxide, blown clean with tap water and distilled water, then dried with ether and sharpened before autoclaving with the set.

For phlebotomies in males a No. 13 needle is recommended; in females the No. 15. It is wise to use 2% Novocaine and a No. 24 or 25 Hypodermic Needle for this injection as a routine procedure.

The distilled water for rinsing this apparatus should be prepared in any of the standard types of stills which are equipped with adequate baffles to prevent the carrying over into the condenser of whole drops of water.

The routine tests should be done on this water both chemically and biologically to establish its freedom from pyrogen in the preparation of sodium citrate and isotonic-glucose solution. It is advisable to autoclave these solutions at a low pressure, approximately 6 pounds, for a period of one-half hour or forty minutes

rather than at the higher pressure, about 20 pounds, used to sterilize equipment, in order to prevent discoloring of the citrate solution and caramelization of the glucose solution. It is thoroughly desirable that all fluids intended for intravenous use should be autoclaved within two to four hours after distillation in order to prevent the recurrence of pyrogens.

The Phlebotomy sets should be wrapped in one complete unit including the bottle, stoppers, rubber tubing, needles, syringes for Novocaine and test tubes for Wassermann samples, and autoclaved at a temperature of approximately 254° F. for a period of at least 15 minutes. Empty bottles should be put into the autoclave upside-down unless a small amount of fluid is layered on the bottom. When needles are inserted into test tubes to prevent damage to the point, the point should be up rather than down in order to prevent collection of air around the tip which might prevent complete sterilization.

#### (6) FLOOR SPACE

Drawing of blood is best done by teams consisting of a director, two nurses and a secretary. It is possible for one doctor and one secretary to function with six pairs of nurses, as though six teams were operating if the proper allotment of contiguous space is made.

There should be allotted approximately 70 square feet of floor space, that is the equivalent of a 7x10' room, for each team which is to operate simultaneously. If two doctors on full time duty are to collect blood from 12 donors in let us say the period of one hour, they would require approximately 840 square feet of floor space for the phlebotomies alone, that is approximately a room 20x14'. In addition there would have to be additional space of approximately 200 square feet for the physical examinations, waiting room space of approximately 50x30', or 1,500 square feet; desk space for receptionist, secretary and two technicians of approximately 150 square feet, making a total of between 2,500 and 3,000 square feet of floor space simply for the collection of blood in a program which might call for 500 bleedings a week. This represents a room of roughly 30x90'. This does not take into consideration the space required for the preparation of bloods, for autoclaving, for preparation of apparatus, or for processing the plasma, or office facilities. These details are presented to point out that a program which contemplates handling 100 or more

donors a day requires a great deal of space in order to function well. Where such space is not available no large program should be undertaken.

#### (7) STORAGE SPACE

Adequate space for storing bottles, tubing, needles, wrapping material, gauze bandages, iodine, alcohol, sphygmomanometers, citrate, saline, Merthiolate, etc., should be given very thorough consideration in setting up any unit for in almost every instance in the experience of this Association the space allotted for storage has proved to be small.

#### (8) REFRIGERATION

The center around which any blood bank or plasma bank operates is the refrigerator. In large scale work it becomes the real limiting factor in the amount of blood that can be drawn and stored in any given location. The small household type of refrigerators used by some of our hospitals in the early days of this project proved utterly inadequate not only because of lack of storage space but because of "cold spots" in the refrigerator, lack of automatic defrosting equipment, lack of equipment for recording temperatures, lack of safety devices for notifying the operators that the temperature in the ice-box varied from that in the compressors and often because of the vibration resulting from the action of the motors.

Some of the larger refrigerators offered great difficulty in moving from one place to another in attempting to find the most suitable working space. The cost of moving such larger units proved to be a factor worth noting. Since this project was started with the idea of eventually being useful in case of a national emergency, it has been thought well to think of refrigeration in terms of units of considerable capacity yet small enough to be mobile, the idea being to increase the units when greater capacity is desired rather than to increase the size of the individual unit. From a discussion of the problem with the engineers of the larger refrigerating companies the following seems to be a fair set of specifications for the construction of a refrigerator suitable for handling blood and plasma in collection centers handling blood up to the number of 1,000 per week:

1. The refrigerator should be constructed of a good grade of white porcelain, monel metal, stainless steel or other suitable



material. The shelves should be adjustable and of reenforced steel. The wire grating of the shelves should have apertures small enough to prevent the tipping of small containers, yet adequate to allow free circulation of cooled air. The shelves should be provided with rubber bumpers in order to diminish the amount of vibration and each door should have a separate lock incorporated into the handle.

2. We recommend a unit of about 50 cubic feet capacity. This allows space for the storage of about 200 containers 1 foot high each holding 600 ccs. and allows shelf space for each container of approximately 36 square inches. The overall depth of the box should not exceed the width of the usual house door of 3 feet. It is suggested that the inside depth be not greater than 2 feet 6 inches, since the average arm reach is not greater than this. The height should not be greater than 6 feet. These dimensions take on considerable importance for the convenience of nurses who are small in size who have to have access to these ice-boxes many times during the day.

3. A cooling unit of the force-convexion type utilizing "Freon" (dichlor-difluoro-methane, F 12) as the refrigerant seems to be the safest and most suitable for this type of work. It should have a high refrigerant temperature, 25-28 degrees Fahrenheit, in order to permit uniform temperature throughout the box and eliminate the possibility of "cold spots".

4. The compressor and motor should have speed enough (1,500 to 2,000 R.P.M.) to insure smooth action and freedom from vibration. Vibration in any form causes more rapid disintegration of the blood. In addition to the fan in the ice-box for circulation of cooled air, ventilation of the compressor unit should be adequate. Refrigerators designed to be used in warm climates would profit by additional outlets in the front and sides of the refrigerator in order that free circulation of air may be obtained. A one-third horsepower unit in such a refrigerator should have a capacity of roughly 3,000 B.T.U. per hour.

5. Temperature control should be regulated by a built-in thermostat to insure constant temperature and continuous automatic defrosting. The temperature should be maintained at 38 degrees Fahrenheit (3-4° C.) and within a range no greater than plus or minus 1 degree Fahrenheit. If the temperature is to be limited to a range of less than plus or minus 0.5 degrees Fahrenheit

it is suggested that two compressors be installed, since a single machine working within a very closely limited range does not have sufficient off cycle time to properly defrost the cooling unit.

6. A temperature recording device situated within easy view outside of the box should be a part of the standard equipment in order that a permanent record of the performance may be obtainable.

7. An alarm system, either lights or bells, to indicate the temperature shifts above or below the range of the compressor thermostat should be provided for.

8. The installation of an ultra violet lamp is a worthwhile addition to such a refrigerator.

9. Such refrigeration should be obtainable at a cost of about \$15 per cubic foot of capacity.

#### IV

##### Plasma vs. Serum

During the progress of this work two schools of thought have grown up concerning the best available blood substitute. On one side there are the proponents of plasma, on the other side the proponents of serum. The reports of clinical trials with plasma far outnumber those with serum, chiefly because many of the "blood banks" remove the plasma from stored blood at the end of a few days or a week.

From all available information plasma used at a relatively early date after its removal from the blood is innocuous. When stored for long periods of time however several disadvantages appear, namely, the growth of chance contaminants to dangerous proportions during storage, the tendency for the protein content to precipitate out making it unsuitable for emergency use unless filtered and the difficulty in filtering it through earth, porcelain or asbestos filters.

On the other hand the adherents of serum have approached the problem from a background formed by years of preparing immune or convalescent's sera. The amounts used have been small as a rule.

In an attempt to arrive at some definite conclusion data was sought from many sources with the hope that its tabulation would give this Association more definite grounds for making a decision one way or the other.

Notes were compiled from articles, letters or verbal messages from many sources. Dr. Charles H. Best, of Toronto, has been good enough to pass on to us all the information in his files concerning this problem. The feeling is that it should be settled one way or the other at the earliest possible date.

The following are chronologically arranged:

April, 1940—Strumia, Wagner and Monaghan (Annals of Surgery 111:627):

*"Conclusions:* The intravenous use of citrated blood plasma without cross-matching is both safe and convenient. This applies to fresh plasma, or plasma preserved by either refrigeration at 4° C. or the Lyophile process. Serum, separated after clotting, may cause reactions, often severe, when intravenously injected, whether employed fresh or preserved by either refrigeration or the lyophile process."

May, 1940—Scudder (Annals of Surgery 112:514):

"Plasma approaches the ideal physiologic perfusion fluid and is superior to acacia, glucose, salt and serum. Unrefrigerated plasma, lyophilized serum and autopsy plasma appear definitely abnormal."

May 24, 1940—From: Department of Pathology, University of Cambridge. Dr. A. N. Drury, Chairman of the Committee on Traumatic Shock and Blood Transfusions of the Medical Research Council of Great Britain:\*

"Dear Dr. Best:

Thank you for sending me the concentrated serum.

Opinion is divided here on the question of liquid citrate plasma from the blood bank as against dried serum. It is obvious that both have their place but that will hold up the form officially adopted by the services a bit.

In view of the American reports on the reaction obtained with dried serum, I send you the results of some 94 injections which show that our product is pretty innocuous.

Reactions following dried serum transfusions in 94 cases. The figures in brackets throughout refer to those who had no reactions:

\*NOTE:—In all quotations from letters appearing in this report only the extracts dealing with the subjects of plasma and serum are given and no effort has been made to indicate the places therein where portions dealing with extrinsic subjects have been omitted.

# *Distribution in the cases transfused*

	R.	No. R.	Severity of Reaction	
1. Normals .....	4	(15)	Very mild or mild...	7
2. Acute Haemorrhage	3	(8)	Moderate .....	4
3. Shock .....	2	(28)	Severe .....	3
4. Burn Shock .....	0	(5)		
5. Nephrosis .....	1	(7)		14
6. Sick but not requiring transfusions	4	(17)		
	14	(80)		
	94			

# *Character of Reaction*

Headache .....	2
Nausea .....	1
Vomit .....	4
Shivering .....	2
Vomit Rigor and Temp. ....	2*
Lumbar Pain .....	2
Urticaria .....	1†
	14

\* Both of these were due to infected serum.

† Protein sensitive individual. Otherwise cause of reactions unknown.

Slight rises of temperature alone have not been included, but two such rises occurred.

# *Relation of dosage to reaction:*

	R.	No. R.
Four times normal concentration 10 cc...	1	(0)
20 cc...	1	(4)
50 cc...	4	(35)
100 cc...	6	(22)
150 cc...	1	(4)
200 cc...	0	(3)
300 cc...	0	(1)
600 cc...	0	(1)
Twice normal concentration... 100 cc...	0	(2)
200 cc...	0	(2)
300 cc...	0	(0)
400 cc...	0	(3)
Normal concentration ..... 400 cc...	1	(1)
600 cc...	0	(2)
	14	(80)

14 reactions in 94 injections..	15% (approx.)
7 mild reactions .....	7.5% "
4 moderate .....	4.2% "
3 severe .....	3.2% "
80 no reactions in 94 injections	85%

## General Remarks:

1. The observers using the dried serum were asked especially to look out for any reaction, so that it may be safely concluded the "reactions" were not missed.

2. The dose recommended was 50-100 ccs. of a four times concentration; this explains the large number of cases receiving these doses.

*Effect of dried serum transfusions on "Shock" and Acute Haemorrhage Cases*

Traumatic Shock .....	15 cases
Post Operative Shock .....	8 "
Acute Haemorrhage .....	11 "

Total..... 34 cases

*Condition After Transfusion*

	Traumatic	Post Operative	Acute Hem.
Unchanged .....	6	1	3
Improved .....	6	3	7
Greatly improved .....	3	4	1
Worse .....	0	0	0
	<u>15</u>	<u>8</u>	<u>11</u>

## Detailed Analysis

*Condition Unchanged by Transfusion*

*Condition before transfusion*

	Traumatic	Post Operative	Acute Hem.
Moderate .....	1	0	3
Poor .....	2	1	0
Desperate .....	3	0	0
	<u>6</u>	<u>1</u>	<u>3</u>

*Condition Improved by Transfusion*

*Condition before transfusion*

	Traumatic	Post Operative	Acute Hem.
Moderate .....	1	1	3
Poor .....	4	3	4
Desperate .....	1	0	0
	<u>6</u>	<u>4</u>	<u>7</u>

*Condition Greatly Improved by Transfusion*

*Condition before transfusion*

	Traumatic	Post Operative	Acute Hem.
Moderate .....	0	0	0
Poor .....	3	1	1
Desperate .....	0	2	0
	<u>3</u>	<u>3</u>	<u>1</u>

*Effect on Blood Pressure*

	Traumatic	Post Operative	Acute Hem.
Unchanged .....	5	1	4
Raised .....	10	7	7
Lowered .....	0	0	0
	<u>15</u>	<u>8</u>	<u>11</u>

*Effect on Pulse Rate*

	Traumatic	Post Operative	Acute Hem.
Unchanged .....	8	5	7
Slowed .....	3	3	4
Enhanced .....	4	0	0
	<u>15</u>	<u>8</u>	<u>11</u>

*Effect on Blood Dilution*

	Traumatic	Post Operative	Acute Hem.
Unchanged .....	0	0	0
Diluted .....	6	3	8
	<u>6</u>	<u>3</u>	<u>8</u>
	6 only tested	3 only tested	8 only tested

## General Remarks:

1. In some cases the serum transfusion was combined with saline, whole blood transfusion or the administration

of high concentrations of oxygen. The results refer to the immediate effect of the transfusion. As the number of cases treated is so small it would be useless to divide them up into cases receiving serum alone, and those combined with other transfusions.

2. Some of the cases owing to the severity of the trauma, etc., could be considered to be unlikely to improve with any treatment."

At a later date Dr. Best received the following letter from Dr. Omond Solandt:

Nov. 3, 1940—From: Ministry of Health Emergency Hospital Scheme, S. W. London Blood Supply Depot, Benhill Ave., Sutton, Surrey.

"Dear Dr. Best:

Your letter was especially timely because the increasing trouble with the clotting of plasma after filtration and with infection in unfiltered plasma is leading to the idea that plasma may be scrapped in favor of serum, preferably dried serum. Both Drury and Proger are in favor of the idea if more data on the safety of serum can be collected. We are at present getting a stock of filtered liquid serum in order to give it a thorough trial. I think that the usefulness of dried serum is now well established.

Our observations on cases of shock have necessarily been rather limited but have led to the conclusion that the average air raid casualty does not show haemoconcentration and is better treated with blood than with plasma or serum. There are a few cases of crushing injuries and of burns where there is very marked haemoconcentration and serum is the only thing. This does not mean that we have no need of serum or plasma. Their place in our scheme is to cut down the wastage of blood. We can give all the hospitals a stock of serum or plasma and then deliver blood to them when they have casualties. They can start treatment with the serum or plasma before the blood arrives. This scheme is working very well and is keeping wastage down very well.

One of the most interesting problems is the incidence of kidney failure after shock. There have been many cases most of whom had blood transfusions. The anuria was sometimes blamed on the transfusion though there was no other evidence of incompatibility. We have one case just now in which no transfusion was ever given as the injuries did not seem severe. It will be a week tonight since she was injured and the total urine output is under 300 cc.

The blood urea was 325 yesterday in spite of every sort of treatment."

*General Observations by American and Canadian Doctors:*

The following excerpts give the substance of opinions voiced at the Annual Meeting of the American Human Serum Association, June 10, 1940. No effort has been made to quote verbatim:

Dr. Max M. Strumia, Bryn Mawr Hospital, Bryn Mawr, Pa.:

It is most important to keep well in mind the difference between serum and plasma. By serum is meant the liquid phase of blood separated after clotting has taken place; by plasma is meant the liquid phase separated from the blood without previous clotting which is usually prevented by the addition of sodium citrate. It is in the process of clotting that toxic properties are imparted to the serum.

I have been interested in this problem intermittently since 1927, at which time human serum was administered intravenously in cases of severe infections, especially those of streptococcic origin. It was noted then that intravenous injection of serum in sufficiently large quantities was commonly followed by reactions, often severe, even when the sera was homologous, that is, did not cause agglutination of the recipient's erythrocytes. In 1929 citrated blood was centrifuged and the citrated plasma was used in place of the serum. It was then noted that plasma, even when intravenously injected in large doses, caused no reactions. This freedom from reactions has been proved by over 1800 administrations of plasma thus far given, of which over two-thirds were given intravenously.

Dr. John Elliott, Salisbury Pathological Laboratory, Salisbury, N. C.:

The successful use of stored dilute human plasma as a substitute for whole blood as suggested in 1936 is reported. Administration of 482 plasmas, 53 of which were stored at room temperature for periods of from one day to two hundred and fifty-two days, is discussed. Only three reactions, all typically pyrogen, occurred. These plasmas were administered intravenously, subcutaneously and intramuscularly in some conditions with equal apparent benefit.

Dilute plasma prepared by our method is an effective substitute for whole blood. It is easily prepared. It is adaptable to all institutions. It has been administered safely and effectively after storage for periods up to nine months.

Clinical use after storage under widely varying conditions, both in the home and in the hospital, indicate that it is a valuable and safe therapeutic agent.

On November 13, 1940, in a personal communication Dr. Elliott had this to say:

The use of the Transfuso-Vac and Centri-Vac developed in this laboratory so simplifies the collection of blood that I can draw from six to eight 500 cc. quantities of blood with the aid of two nurses or technicians in thirty minutes. But more important than that, is the record of complete asepsis. I have prepared more than 1,000 plasmas, several hundred without a chemical preservative, without a single contamination. The additional expense involved may seem great but when the total cost of preparing the citrate solution, purchasing the necessary equipment, the additional force necessary to collect blood and prepare equipment, and the loss of plasma from contamination is considered, it should be inconsequential.

It is true that pooling plasma reduces the titre of agglutinin. It is also true that it is safe to administer from high titre incompatible plasma. My animal work indicates that unbelievably huge quantities of incompatible agglutinins can be administered rapidly with safety.

From my work it is becoming evident that 500 cc. of plasma can be preserved in 100 cc. of 10% or 20% dextrose in Physiological Salt Solution without formation of fibrin or sediment.

Dr. Sidney O. Levinson, Director, Samuel Deutsch Convalescent Serum Center, Michael Reese Hospital, Chicago:

Human serum as a blood substitute has been mentioned many years in the past, but it has been applied only recently. The merit of serum or plasma as a blood substitute has been stressed in a number of recent reports. This report on the use of human serum as a blood substitute in forty-seven patients for a variety of medical and surgical conditions, completely confirms our experience with human serum in animal experiments.

The advantages of human serum as a blood substitute have been pointed out in other publications. These advantages may be summed up in a statement that serum can be prepared in large amounts, can be stored over a long period of time without deteriorating, can be shipped and handled without difficulties, and can be administered with complete safety without preliminary typing and compatibility tests. Human serum or plasma should prove a very

important adjunct in any hospital transfusion service in the future.

Personally I hold no brief for either serum or plasma. Possibly plasma can be more easily prepared but on the other hand, serum is a clearer and better product. No fibrin veil reactions with either one. Careful laboratory tests for sterility should be performed at all times to control the safety of the product.

On November 18, 1940, in a personal communication, Dr. Levinson had this to say:

We have given over 200 serum transfusions in the last few months, the amounts ranging from 250 cc. to 1500 cc. The average dose has usually been around 500 to 750 ccs. There have been only two reactions and they consisted of urticaria which was not severe.

There are not great differences between plasma and serum as transfusion fluids. In our experience plasma cannot be kept over a long period of time because fibrinogen gradually precipitates out in the form of fibrin veils and granular precipitate and requires filtration or centrifugation and clearing before administration. After most or all of the fibrinogen has precipitated out, the solution consists essentially of serum diluted with sodium citrate. If the original intention of the laboratory is to prepare either serum or plasma, we believe that the former is preferable because after its preparation and clarification by filtration it can be stored indefinitely retaining its clear state.

Dr. William Thalheimer, Manhattan Convalescent Serum Laboratory, New York:

We have prepared for a number of years large amounts of normal human serum and different types of human convalescent serum. This serum has not been frozen and dried, but has been kept in the liquid state in the refrigerator at 5° Centigrade. This serum has all been filtered through Berkefeld filters, and is clear and without any tinge of hemoglobin. These different serums have been administered by many physicians intravenously for therapeutic purposes in both small and large amounts, and some children have received doses which would be the equivalent of 300 or 400 cc. for a 150 lb. adult. Hundreds or possibly thousands of injections have been made. Most times the serum has been given relatively quickly, that is, in from 5 to 15 minutes. There has been a disastrous reaction, and fever and chill reactions have been extremely uncommon, not only oftener than experience has shown will occur in a

similar number of intravenous injections of physiological saline solution or 5% glucose solution. Other human serum laboratories scattered over the U. S. have had the same experience with human serum which they prepared and distributed.

Dr. Stuart Mudd, Professor of Bacteriology, University of Pennsylvania, School of Medicine:

I wish we could all do comparative studies. A really adequate and careful comparison of serum vs. plasma should be made. In the meanwhile plasma seems to be the most satisfactory, at least for intravenous use. A lot of careful people do and some others do not get reactions with the use of serum. For emergencies I think we ought to prepare plasma for the treatment of shock.

Nov. 12, 1940—Dr. Harry E. Foster, Medical Director, The Cutter Laboratories, Berkeley, California:

I would say that besides scrupulous asepsis in obtaining the blood and preparing the serum or plasma, we use both Berkefeld filtration and preservation with Merthiolate 1:10,000. I cross my fingers when I say that so far we have had no trouble with contamination. I presume there are no preservatives which when used in concentrations safe for human administration will not permit the growth of some occasional resistant organisms. The high bacteriostatic effect of mercurial preservatives always leaves one with the feeling that something may have slipped by in a test.

I know the feeling among some workers is that serum is definitely more toxic than plasma. So far as our own experience goes we have a report of only one severe reaction and that was similar in character to pyrogenic reactions which frequently follow the intravenous mass injections of saline.

The human serum which we market is prepared from clotted blood. We have been experimenting with methods designed to remove fibrin from the fluid portion of the blood separated from the cells.

Nov. 15, 1940—Dr. J. C. Meakins, Professor of Medicine, McGill University:

We have used concentrated serum prepared by Dr. Best in Toronto on 15 cases and in 5 of them (33%) we have had serious reactions and we do not feel justified in continuing to use it, at least at present. During the same time we have used plasma on many more cases and have

had no reactions except urticaria. It is true that the plasma has not been concentrated but I cannot believe this would have made any difference. We hope in the near future to have an opportunity of using concentrated or dried plasma.

Nov. 18, 1940—Dr. I. S. Ravdin, Professor of Surgery, University of Pennsylvania School of Medicine:

It has been our opinion that plasma is safer than serum. We base this upon the fact that we have had many more reactions following the use of serum than when plasma was used. We have seen no differences in the therapeutic efficacy of serum over plasma.

The most authentic word to date is contained in a letter written on October 31, 1940, by Sir Edward Mellanby, Chairman of the Medical Research Council of Great Britain, to Dr. P. D. Wilson, Director of the American Hospital Unit at Basingstoke, England, which Dr. Wilson sent to Dr. Rhoads. In this letter it was said:

"The preference is for dried serum. The reasons for this are:

- (I) that serum can be bacteriologically filtered through Seitz pads;
- (II) that the dried product keeps indefinitely;
- (III) that if a chance organism gets in during the process, there is no opportunity for this to grow and produce toxins;
- (IV) it can be produced without the addition of an antiseptic;
- (V) there is no evidence that it is a 'toxic fluid'.

On the other hand citrate plasma has the following disadvantages:

- (I) it clots after filtration through Seitz pads;
- (II) if it is issued without being filtered, it is opalescent and subsequent infection cannot be detected;
- (III) that there is a suspicion that after some time the antiseptic action of Merthiolate dies out, and that organisms if present begin to grow;
- (IV) that reports are coming in of brisk reactions after plasma, due presumably to using infected plasma.

During the air raids on London during September, it may be assumed that approximately 1,500 casualties were transfused, the majority with plasma. The amount given to each patient varied from 1,000 cc. to 2,500 cc."

Nov. 27, 1940—The following cable from Dr. Omond Solandt in London was received by Dr. C. H. Best, Toronto:

"Cambridge dried serum used 92 cases with nineteen mild reactions. Mostly four times normal concentration, dose 50 to 500 cc. Some normal concentration, dose 300 to 1200 cc.—20 cases with filtered plasma gave six mild reactions—no official view likely soon. Liquid serum being tried."

Dec. 4, 1940—Letter to Professor C. H. Best from Dr. A. N. Drury:

"The Canadian Red Cross asked the other day if they could supply us with liquid plasma. I replied that it would be much better if they sent us a dried product, and put the whole matter under your charge. I hope that was not distasteful to you, but I felt that I could rely upon anything you took up. We have had good results with serum, the reactions in 202 reported transfusions being 34, practically all mild or very mild. We stick to serum as it is so much easier to keep sterile than plasma, can be Seitz filtered easily, and you get more protein for the same volume of fluid withdrawn. I shall send you a detailed account of our process at Cambridge.

I am wondering how you are going to handle your dried product in bulk and how you are going to pack it. Experience has taught us that 400-500 ccs. is the smallest dose likely to do good in shock with severe trauma, so that would make a good unit, (better than ours which is 200 ccs.).

The question of bulk handling is not easy if sterility is to be maintained. Units are established for bacterial filtration of serum and plasma, so that we can now offer whole blood, dried serum, liquid serum and liquid plasma, the last three Seitz filtered. I see no evidence to suggest that there will be any difference between the clinical value of dried serum, liquid serum and liquid plasma, and unless anything unforeseen turns up, I believe liquid and dried serum will prove to be our main stand-by. The advantage of liquid plasma which has been so often stressed, namely that it can be got from the blood banks, seems to be of little import in the stress of air raids. We keep our blood banks as low as possible, try to give the hospitals an emergency supply of liquid

plasma and tell them to ring up for blood from the depots as they want it. Liquid serum is just undergoing a clinical trial, but as air raids on London have been mild lately, we have not had much of a chance to try it, but on the few cases it has been tried, it has done just as well as plasma."

#### SUMMARY

(1) Plasma, when free from clots and uncontaminated, is a safe, effective blood substitute but it is very difficult to process through bacterial filters. Without bacterial filtration the danger of latent contamination in plasma which has been stored for any length of time should not be overlooked.

(2) Evidence is increasing to sustain the contention that serum, properly prepared, is an innocuous and effective blood substitute.

(3) Plasma has been prepared throughout this project but further evidence is needed to establish the relative merits of these two blood substitutes.

#### V

##### Dried Plasma and Serum

At the very outset of this project it became clear that, though liquid plasma in large quantities was the immediate aim, the need of dried plasma or serum would perhaps receive the chief attention of the Association in the very near future. This it was felt would be so because of the advantages of a blood substitute in a dried form which had been pointed out by several investigators. These may be summed up as follows:

1. More complete stability of the material for longer periods of time.
2. Less likelihood of chance contaminants, multiplying to dangerous proportions during storage.
3. Less weight, and the possibility of compressing powder, therefore less storage and shipping space necessary.
4. Less need for refrigeration even in very warm climates.
5. Its ready adaptability for use as hypertonic, isotonic or hypotonic solutions upon reconstitution with sterile distilled water.

To Ehrlich, perhaps, goes the credit for pointing out the increased stability of serum in dried form. Martin (Journ. Path. and Bact. 3:507, 1896) has described an apparatus for the dessication of serum from the liquid state, while Shackell (Am. J. Phys. 24:325, 1909) first presented the method of drying biological products from the *frozen state*.

The present revival of interest in this thirty year old process may be traced to an attempt of Elser (J. Immunol 28:433, 1935) to set up in the Mulford Laboratories an apparatus capable of drying larger quantities of serum than he had previously been able to do in his own laboratory at Cornell. This work was abandoned after a while by Elser but Dr. J. Reichel continued the investigation at the Mulford Biological Laboratories.

When in 1933 Dr. Reichel had difficulty in solving the problem of handling powdered "Lyophile" serum, he went to Dr. Stuart Mudd, of the Department of Bacteriology in the Medical School of the University of Pennsylvania, for aid and it is from this laboratory in the years that have followed that the most important contributions concerning the drying of plasma and serum have come. Practically the whole subject may be found covered in three articles by Dr. Earl W. Flosdorf and Dr. Mudd. The first of these, "Procedure and Apparatus for Preservation in 'Lyophile' Form of Serum and other Biological Substances" (J. Immunol. 29:389, 1935); the second, "Improved Procedure and Apparatus for Preservation of Serum—Cryochem-Process" (J. Immunol. 34:469, 1938); and finally "The Desivac Process for Drying from the Frozen State" (J. A. M. A. 115:1095, 1940).

The basic principle in each of these processes is the same and consists of rapid freezing of the blood substitute at a very low temperature and the rapid dehydration from the frozen state under high vacuum. In the "Lyophile" process condensation is brought about by freezing the sublimated water vapor; in the second process the water vapor is absorbed by a chemical, in most instances calcium sulphate, and in the third, the "Desivac Process," water vapor is removed directly from the vacuum chamber by an oil sealed pump and discharged to the atmosphere in the liquid phase, thereby doing away with the necessity for low temperature condensing or chemical dessicants.

The machinery to carry out any one of these processes is rather expensive. A "Lyophile" machine capable of drying 7 liters of plasma in sixty hours costs in the neighborhood of \$1,000. The

equipment necessary to dry a comparable amount by the "Cryochem Process" costs even more. The Desivac apparatus designed to dry approximately 25 liters of plasma each 24 hours costs approximately \$5,500. It was created to a large degree with the idea of handling large volumes of material.

The simple procedure advocated by Edwards, Kay and Davie (Brit. Med. Jour. 1:377, 1940) has been tried out and as a laboratory procedure functions moderately well.

Another apparatus of a small laboratory type which is just a modification of one of the earlier methods of drying is that made by Harper, Essex and Osterberg of the Mayo Clinic (Pro. Staff Meetings 15:689, October 30, 1940). This apparatus has several faults from the point of view of mass production but apparently works quite well in the laboratory.

The "Adevac Process" of Hill and Pfeiffer (Ann. Int. Med. 15:201, 1940) is another attempt to arrive at a simple, cheap method of preparing plasma. F. W. Hartman (J. A. M. A. 115:1989, 1940) has reported the construction of an experimental unit for drying which utilizes a somewhat different principle from those described above. In essence it consists of simply concentrating the plasma or serum by placing it in double-walled cellophane cylinders and blowing warm air on them from a fan until complete dehydration is accomplished.

One of the best functioning units built on the old "Lyophile" process principle, but eliminating some of the difficulties of uniform distribution of warm air around the pre-frozen ampoules, is that now in operation at the Rockefeller Institute for Medical Research, where it is being used to dry the influenza vaccine under the direction of Dr. Goodner and Dr. Horsfall.

For immediate use, the plant of Sharp & Dohme Company in Philadelphia, has the largest available set of drying apparatus and should plasma or serum be prepared in large quantities in the larger civilian centers of the East, it might well be shipped to this plant for drying. This plan, however, is not as desirable as one which would collect the blood, prepare the plasma or serum and dry it in single units in one laboratory for each particular center.

Because the high initial costs of the various types of drying apparatus would become a very large item in equipping a laboratory for quantity production, Dr. Rhoads of the Memorial Hospital assigned Mr. Theodore Folsom, a member of his staff in the Physics



Department, to the problem of creating a drying machine which could handle moderately large quantities of plasma at a lower cost than machines on the market. To a great extent, Mr. Folsom has accomplished this purpose. With a single unit of the apparatus which he has constructed, 250 cc. of plasma may be dried each 24 hours and the parts of such a unit are stock material in any laboratory.

The following estimate has been submitted by Mr. Folsom of the costs of such a unit and of 10 unit, 50 unit and 100 unit Plasma Drying Stations: Such prices are what must be allowed for first installation and include manufacture of all parts, but no installation labor.

Cost of one *unit*, exclusive of vacuum system, filling accessories, etc.:

1 Thermos Bottle, 4 l. straight wall.....	\$5.70
1 Glass condenser (Hopf Glass Co.).....	3.75
1 Filling needle (12" long, No. 13 with socket).....	.75
2 Small skirted stoppers (E&A) @ .10.....	.20
1 Solid No. 6 stopper.....	.05
1 Special No. 6 stopper with skirt.....	.20
1 One Ft. rubber tubing $\frac{5}{16}$ I.D., $\frac{3}{8}$ O.D.....	.15
1 Clamp to hold condenser (E&A No. 20218).....	.45
1 Clamp, knuckle (Fisher).....	.30
1 Spring pinchcock (E&A No. 20302).....	.12
1 Screw pinchcock (E&A No. 20294).....	.22
Total Unit Cost.....	\$11.99

#### *Cost of 10 Unit Installation*

Cost of pumping system, manifold, gauges, etc. for ten units, or "decade":

Cost of manifold like ours, with mounting for table use .....	\$25.00
(Note: Added convenience could be obtained if valves were added to manifold coiling, an additional \$35.00)	
Hyvac pump and motor, mounted.....	75.00
Simple vacuum gauge (discharge tube type).....	8.00
"Air Bath" Cabinet, heated by light bulbs (in unfinished pine wood) .....	25.00

Total pumping system for decade..... \$133.00

Cost of ten drying units like above, itemized.....	\$119.90
Complete cost of ten unit Dessicator (minus filling accessories) .....	252.90
Same, except manifold has valves added for convenience as above suggested .....	288.00

#### *Cost of 50 Unit Drying Laboratory*

Accessories for rapid handling of plasma:

Pre-freezing machine to freeze four ampoules at one time .....	\$100.00
Dry ice storage tank (simple insulated box for 300 lbs. dry ice) .....	20.00
Tools: flaming off torch .....	6.00
oxygen tank valve .....	15.00
hose for torch .....	1.00
dry-ice-hammers, etc. ....	5.00
5 Autoclave racks for sterilizing and handling glassware .....	25.00
2 filling racks for sterile filling of ampoules.....	4.00
1 Aspirator pump for filling units rapidly.....	19.00

Total accessories of 50 unit laboratory..... \$195.00  
(Note: This is almost the same for 100 unit laboratory)

50 unit simple drying equipment @ \$252.90 for each 10 units (as above) .....	\$1,265.00
Total cost of 50 unit station.....	1,460.00

#### *Cost of 100 Unit Drying Laboratory*

Accessories as above .....	\$ 195.00
100 simple units .....	2,529.00

Total cost of 100 unit laboratory.....\$2,724.00

(Note: because of large volume of glass handled, some additional accessories might be needed in the larger unit.)

#### *Summary*

In the early days of the use of dried plasma and serum there were many reports of severe reactions following their use. This was particularly true of dried serum. The reactions were less marked with dried plasma. During the last year there have been increasing reports of the use of dried plasma and serum without reactions

both in this country and abroad, yet the feeling persists among those who know the problems best that more clinical evidence and more experimental data concerning the changes in such dried material should be massed and reviewed before any large scale production of either dried serum or plasma should be attempted. With the gradually improving methods of preparation the reaction rate is decreasing, but this subject cannot be considered at this stage to be completely understood and the production cannot be recommended with full assurance that no harm will follow its use.

## VI

### Research

This project was begun with a full realization that the methods for collecting blood on a large scale, and preparing plasma or serum were not thoroughly worked out and that there were considerable differences of opinion as to the most effective methods of doing each of the many steps in such a process. But it was felt that enough was known to assure a safe and usable if not ideal blood substitute, and that, because of the urgency of the need, a start should be made with the knowledge at hand. It was agreed that during the actual operation further investigations should be continued for the purpose of improving the technique, standardizing routines, and solving some of the many problems which clearly presented themselves for the first time only after an attempt had been made to institute factory procedures.

Among the first pieces of experimentation delegated and financed by the Association was that of determining the most suitable container for the collection of blood. For this purpose certain funds were made available to Dr. John Scudder, of the Presbyterian Hospital, for redesigning and testing the dumb-bell bottle. Funds were made available to Dr. Lester J. Unger, of the Post Graduate Hospital, in order that he might investigate the problems associated with creating a bottle which could be used for centrifugation with maximum yields of plasma or as a sedimentation bottle with a reasonable yield of plasma.

#### APPARATUS FOR CONTINUOUS CLOSED SYSTEM

One of the first real problems to present itself was that of the aseptic collection of blood, pooling of the plasma and transference

of the pooled plasma to the final containers. Discussions were had with the representatives of the various liquid supply houses and glass companies such as the American Hospital Supply Corporation, the Hospital Liquids Company, the MacAlister, Bicknell Company, the Wheaton Glass Company, the Cutter Laboratories, the Libbey-Owens-Ford Glass Company, and the Corning Glass Company, in an effort to have created containers more suitable for the job at hand than those available on the open market.

No system or set of containers had been devised for just such a program. Therefore it became of paramount importance to create or have created such apparatus.

In addition to the experiments related to bottles Dr. Unger was allotted funds for the development of a double needle donor set which would obviate the necessity for ever removing the rubber stopper from the container in order to put in or remove fluid.

#### DRIED PLASMA

When Mr. Folsom's research in developing a less expensive apparatus for this purpose had been carried to a stage which made it seem reasonably probable that it would be successful, funds were allotted by the Association, from those set aside for research, to carry out this project on a larger and more complete scale.

#### PLASMA *vs.* SERUM

When it became obvious that there was some doubt in the minds of many as to whether plasma or serum is the more suitable substitute, as recorded in the opinions above collected in the section, Plasma *vs.* Serum, it seemed wise to set up a rather large scale experimental program to determine the relative toxicity and clinical efficacy of plasma and serum in their liquid and dried forms.

A protocol was prepared, was approved by the Board of Medical Control, and funds with which to carry out the research were approved by the Research Committee and Board of Trustees. It was as follows:

#### PROTOCOL OF EXPERIMENT RELATED TO THE CHOICE OF PLASMA OR SERUM

To determine the relative chemical composition, therapeutic efficacy and toxicity of citrated blood plasma, serum from clotted blood, serum from citrated plasma, dried plasma, dried serum from clotted blood and dried serum from citrated plasma treated with calcium salts to precipitate the fibrinogen.

## I. DONORS

To be supplied by the American Red Cross  
through Captain Scully and Colonel Booth

## II. COLLECTION OF BLOODS

To be done at Memorial Hospital—Dr. Rhoads

(1) From each of a series of 500 cc. phlebotomies, collect two samples as follows:

- (a) 250 cc. plus 25 cc. of 5% citrate;
- (b) 250 cc. clotted blood

(2) From each of another series of donors, collect two 250 cc. citrated samples, one to be treated with calcium chloride of gluconate to create serum.

## III. PROCESSING AND POOLING OF PLASMA AND SERUM

To be done at the Manhattan Convalescents' Serum Laboratory—Dr. Thalhimer. All bloods to be centrifuged, cultured, filtered and pooled. Pools of

---

LIQUID  
Plasma  
Serum from Blood  
Serum from Plasma

---

## 1. Chemical Tests

Dr. Weech, Babies Hospital—Protein Content before and after drying.  
Mr. Moore, Columbia University—Plasma and Serum Tiselius Patterns.  
Dr. Scudder, Presbyterian Hospital—Electrolytes.

## 2. Biological Tests

Dr. Chambers, New York University—Observation of Capillaries following Perfusion with Plasma and Serum

## 3. Clinical Trials

Presbyterian Hospital—Dr. Self—6 types of material 3 Liquid—3 Dry. Data: Type of Case; Wt. of Patient—(App. Cir. Vol.) Hematocrit & Protein Before and After—Reactions.

## IV. DRYING OF PLASMA AND SERUM

Memorial Hospital, Dr. Rhoads and Mr. Folsom (Lyophile Process)—

Data: Water Content—Yield—Volume comp. to Liquid—Rate of Drying Capacity of Units Cost. Material to be supplied by Dr. Thalhimer.

---

DRIED  
Plasma  
Serum from Blood  
Serum from Plasma

---

## 1. Chemical Tests

## 2. Biological Test

## 3. Clinical Trial

The question naturally arises as to why this particular set of data was considered important.

The answer may be best arrived at by a short discussion of the various steps outlined above.

*Shifts in Protein Components*

(1) The osmotic pressure exerted by any infusate of plasma or serum is to a large degree the resultant of its protein content. This content then must be determined in order to establish a base line upon which the clinical response may be represented as a function of the proteins introduced.

Furthermore, certain changes in the form and content of the various protein fractions are known to take place when plasma or serum are desiccated. These changes must be accurately measured if differences in biological and clinical responses following infusions of the same substance are to be explained on the basis of these changes.

*Electrophoretic Patterns*

(2) Straight chemical analysis, it has been shown, does not represent a method sensitive enough to record small quantitative shifts in proteins, so other methods have been and are sought to analyze more critically the protein components of biological substances.

Tiselius (Upsala, Almquist and Wiksells Bottryckeri, 1930) introduced the moving boundary method of studying the electrophoresis of proteins and demonstrated that by this means the nature and stability of proteins in plasma could be determined much more accurately. Applied to the study of changes in preserved blood, it offers infinite possibilities.

For more information concerning the method the reader is referred to the writings of Longsworth, Shedlovsky and MacInnes (Jour. Exp. Med. 70:399, 1939) and Scudder (Ann. Surg. 112: 502, 1940). Let it suffice here to say that this seemed a golden opportunity not only to study the stability of proteins but also to correlate the various electrophoretic patterns with the results found by chemical analysis and clinical trial.

*Electrolyte Studies*

(3) Experimental work financed by the Blood Transfusion Association has already shown the importance of the shifts in inorganic salts (Scudder, Drew, Corcoran and Bull, J. A. M. A., June, 1939). The electrolyte changes, especially potassium and sodium, serve as an index of changes in the whole blood in a manner

unapproached by previously accepted criteria, such as the degree of hemolysis. Such studies made on samples before and after drying, and on the recipients' plasma after infusions, will throw more light not only on the changes which result from processing but also, perhaps, on the nature of post transfusion reactions.

#### *Capillary Perfusion for Study of Blood Substitutes*

(4) Micromanipulative investigations on the mesenteric blood capillaries have indicated that responses of the capillaries to perfused fluids and to micromanipulation can serve as a bio-assay method in determining the adequacy of various blood derivatives and substitutes which might be used in transfusion or in the treatment of shock. In investigations on the blood capillaries two hitherto unsuspected conditions must be taken into account. One is the factor of muscular capillaries which continue from the terminal arteriole directly into a venule and true capillaries which are off-shoots of the muscular capillary (Zweifach, A. J. *Physiol.*, 120:23, 1937; *Anat. Rec.*, 73:475, 1939). The second is that an adequate distribution of the perfusate through the capillary bed requires the presence of particulate matter in suspension. In its absence the flow is restricted to the muscular capillaries (Zweifach, *Proc. Exp. Biol. Med.*, 14:124, 1940; *A. J. Physiol.*, 130:512, 1940).

The effect of the perfusate may be detected by (1) extent of distribution of the perfusate; (2) tonic effects; (3) varying degrees of stickiness of the endothelium to particles suspended in the perfusate; (4) the development of sticky exudates and intra-capillary thrombi; (5) increased permeability indicated by (a) irregularity in distribution of particulate matter in the stream, (b) close packing of the particles in venous-capillaries, (c) loss of retention of colloidal dyes, (d) extravasation, (e) stasis. The investigation also involves arteriolar contractility and venular responses following the introduction of plasma or serum. Development of edema can be recorded by visual changes in the extravascular regions.

#### *Clinical Trials*

(5) The final answer of course lies in giving the blood substitutes to patients in sufficient quantity to treat the results statistically. To this end Dr. Scudder and Dr. Self have prepared the following form sheets, the data so gathered to be transferred to complementary punch cards in order to facilitate eventual tabulation and evaluation of results.

#### PLASMA & SERUM THERAPY SUMMARY

1-6	NAME .....	WARD.....	UNIT No.....
7-9	DATE TREATMENT STARTED.....SEX male fem RACE White Negro other		
10	DIAGNOSIS .....		
11	AGE specify.....	8	18-23 hrs.
	X Under 1	9	24 hrs. or more
	0-9 Other by decades as given	17	TIME BET. SHOCK & THERAPY
12	BODY SURFACE.....sq.M.	1	0-29 min.
	(Mult. code to nearest hundredth sq.M.)	2	30-59 min.
	0.425 0.725	3	60-119 min.
	(Wt in kgm. x Ht in cm. x 71.84)	4	2-3 hrs.
	Wt.....lbs. ....kgm.	5	4-7 hrs.
	Ht.....in. ....cm.	6	8-11 hrs.
13	CAUSE OF SHOCK	7	12-17 hrs.
	Y No shock	8	18-23 hrs.
	X Hemorrhage—laceration	9	24 hrs. or more
	0 operative hemorrhage	18	PREDISPOSING CAUSES
	1 postop. hemorrhage	0	Exposure
	2 bleeding into G.I. tract	1	Starvation
	3 bleeding into peritoneum	2	Dehydration
	4 other, specify.....	3	Age
14	Y Operation—head & neck	4	Debility
	X brain or cord	5	Hemorrhage
	0 thorax	6	Infection
	1 stomach, duodenum, pancreas	7	Hypoproteinemia
	2 small intestine	8	Constitutional disturbance
	3 large intestine & appendix	9	Other, specify.....
	4 spleen	19	TYPE OF SHOCK
	5 liver or biliary system	Y	Primary
	6 urinary & reproductive systems	X	Secondary
	7 extremities, skin or breast	1	Relapsing—none
	8 prolonged labor	2	slight
	9 other, specify.....	3	severe
15	0 Trauma—simple	4	Remarks
	1 simple fracture	20	CONDITION OF PATIENT
	2 multiple fractures	X	Rectal temperature—
	3 compound fracture		below 97.6
	4 trauma complicated by hemorrhage	0	97.6-98.6
	5 Burns—1st degree	1	98.7-99.6
	6 2nd degree	2	99.7-100.6
	7 3rd degree	3	100.7-101.6
	8 Dehydration	4	101.7-102.6
	9 Other, specify.....	5	102.7-103.6
		6	103.7 or more
16	TIME BET. CAUSE & SHOCK	21	X Pulse rate—below 70
	1 0-29 min.	0	70-79
	2 30-59 min.	1	80-89
	3 60-119 min.	2	90-109
	4 2-3 hrs.	3	110-129
	5 4-7 hrs.	4	130-149
	6 8-11 hrs.	5	150 or more
	7 12-17 hrs.	6	Pulse quality—normal
		7	weak
		8	regular
		9	irregular

22	X Respiratory rate—below 16	5 Chief physical abnormalities in—head
0	16-20	6 thorax
1	20-28	7 abdomen
2	30-40	8 extremities
3	42-56	9 remarks
4	58 or more	
5	Respiration quality—normal	ADMISSION LAB. DATA
6	deep	28
7	shallow	0 Blood group (internat.)—0
8	irregular	1 A
9	Cheyne-Stokes	2 B
		3 AB
23	Y Mental state—orientated	29
X	confused	Y Red blood count—not done
0	unconscious	1 below 2 million
1	Systolic B.P. (mm. Hg.) 0-39	2 2 million
2	40-79	3 3 million
3	80-99	4 4 million
4	100-119	5 5 million
5	120-149	6 6 million or more
6	150-179	
7	180 or more	30
24	Y Diastolic B.P.—0-19	Y Hemoglobin—not done
X	20-39	1 below 2.9 gm.
0	40-59	2 2.9-5.7 gm.
1	60-79	3 5.8-8.7 gm.
2	80-99	4 8.8-11.6 gm.
3	100 or more	5 11.7-14.4 gm.
4	Pulse pressure—0-9	6 14.5-17.4 gm.
5	10-19	7 17.5 or more
6	20-39	(100%=14.5 gm.)
7	40-59	31
8	60-79	Y White blood count—not done
9	80 or more	X below 6,000
		0 6,000-9,999
25	1 Venous pressure—0-39	1 10,000-19,999
2	40-79	2 20,000—or more
3	80-119	5 Polys.—not done
4	120-199	6 below 75%
5	200-299	7 75-84%
6	300 or more	8 85-94%
		9 95% or more
26	Y State of hydration—normal	32
X	dehydrated	Y Blood S.G.—not done
1	Skin—normal	1 below 1.0400
2	hot and dry	3 1.0450-1.0499
3	cold	2 1.0400-1.0449
4	moist	4 1.0500-1.0549
5	pale	5 1.0550-1.0599
6	flushed	6 1.0600-1.0649
7	Cyanosis—absent	7 1.0650-1.0699
8	slight	8 1.0700 or more
9	marked	33
27	0 Heart—sounds regular	Y Cell vol. % (hematocrit)—
1	poor quality	not done
2	gross irregularity	1 below 20%
3	compensated	2 20-29%
4	decompensated	3 30-39%
		4 40-49%
		5 50-59%
		6 60-69%
		7 70% or more

34	Y Plasma protein. (calc.)—not done	0 serum from recalcified plasma
X	below 4%	1 pooled
0	4%	2 group specific
1	5%	3 diluted
2	6%	4 concentrated
3	7%	5 age (since blood drawn) under 1 wk.
4	8% or more	6 1-3 weeks
5	Urine cells—not done	7 1 month
6	none present	8 2-3 months
7	R B C present	9 4 months or more
8	W B C present	
35	Y Urine albumen—not done	41
0	none	Y Dried—plasma
1	one or two plus	X serum
2	three or four plus	0 serum from recalcified plasma
5	Urine S.G.—not done	1 isotonic
6	below 1.010	3 diluted
7	1.010-1.019	4 concentrated
8	1.020-1.029	5 age (since blood drawn) under 1 wk.
9	1.030 or more	6 1-3 weeks
36	Y Est. total blood vol. (L)—not done	7 1 month
2	below 3 L.	8 2-3 months
3	3 L.	9 4 months or more
4	4 L.	
5	5 L.	42
6	6 L.	1 solubility time—immediate
7	7 L. or more	2 1-2 min.
37	Y Est. total plasma protein (gms) not done	3 3-4 min.
1	below 200 gms.	4 5 min. or more
2	200-299 gms.	6 Source—P.H. blood bank
3	300-399 gms.	7 B.T.B.A.
4	400-449 gms.	8 N.R.C.
5	450-599 gms.	9 other, specify.....
6	600 gms. or more	
38	Y Electrocardiogram—not done	43
1	normal	Y Amount used—none
2	T-wave changes	X 0-249 cc.
3	voltage changes	0 250-499 cc.
4	rhythm changes	1 500-999 cc.
5	conduction changes	2 1000-1999 cc.
6	Q.R.S. changes	3 2000-2999 cc.
		4 3000 cc. or more
39	FLUID THERAPY	5 Time required for injection—0-14 min.
0	Blood—none	6 15-29 min.
1	unmodified	7 30-59 min.
2	citrate	8 60-119 min.
3	fresh	9 120 min. or more
4	1 day old	
5	2-4 days old	44
6	5-7 days old	7 Rate of inject.—less than 5 cc. per min.
40	Y Liquid—plasma	8 5-9 cc. per min.
X	serum	9 10-15 cc.
		Y No. of infusions—none
		1 one
		2 two
		3 three
		4 four
		5 five or more

- 45 Protein content of plasma  
or serum,—  
Y below 5%  
X 5%  
0 6%  
1 7%  
2 8% or more  
3 Total protein given—below  
10 gms.  
4 10-39 gms.  
5 40-59 gms.  
6 60-79 gms.  
7 80-99 gms.  
8 100-149 gms.  
9 150 gms. or more
- 46 Y Mode of therapy—intravenous  
X clysis  
0 Saline—none  
1 isotonic  
2 hypertonic (5%)  
3 5% glucose in saline  
4 other, specify.....  
5 Total amt. saline—0-999  
6 1000-1999  
7 2000-2999  
8 3000 or more
- 47 Y Total Na Cl—none  
X less than 10 gms.  
0 10-19 gms.  
1 20-29 gms.  
2 30-39 gms.  
3 40 gms. or more  
4 Eschatin—none  
5 less than 10 cc.  
6 10-24 cc.  
7 25-49 cc.  
8 50-99 cc.  
9 100 cc. or more
- OTHER THERAPY
- 48 1 Heat  
2 Sedation—morphine  
3 barbiturate  
4 other, specify.....  
5 Oxygen  
8 Other, specify.....  
9 Remarks
- RESULT OF THERAPY
- 49 Increase of rectal temp. of 1°  
or more  
(.....)  
1 ½ hr. after therapy  
2 1 hr. after therapy
- 3 1½ hrs. after therapy  
4 2 hrs. after therapy  
5 4 hrs. after therapy  
6 6 hrs. after therapy  
7 12 hrs. after therapy
- 50 Pulse rate after therapy.....  
Y same  
X lower  
0 higher  
1 Pulse quality after therapy—  
same  
2 improved  
3 worse  
Respiratory rate after therapy.....  
4 same  
5 lower  
6 higher  
7 Resp. quality after therapy—  
same  
8 improved  
9 worse
- 51 Y Mental state—same  
X improved  
0 worse  
Blood pressure.....  
1 same  
2 lower  
3 higher  
4 Venous pressure.....  
5 same  
6 lower  
7 higher
- 52 Y Significant reaction—none  
X Chilliness  
0 Chill  
1 Urticaria  
2 Tachycardia  
3 Dyspnea  
4 Fall in blood pressure  
5 Albuminuria  
6 Hematuria  
7 Olyguria  
9 Other, specify.....
- 53 1 General result of therapy—  
same  
2 improved  
3 worse  
4 died, spec. clinical cause of  
death.....  
5 autop. not obtained  
6 autop. obtained  
9 Remarks

## FLUID BALANCE

INTAKE	Date & Hour .....	
	By mouth .....	
	Intravenous .....	
	Subcut. ....	
	Diet .....	
	Other .....	
	Total ccs. ....	
	Total gms. NaCl .....	
	Insens. loss .....	
	<hr/>	
OUTPUT	Urine .....	
	P.R. ....	
	Vomitus .....	
	Suction .....	
	Ostomy .....	
	Other .....	
	Total ccs. ....	
	Total gms. NaCl .....	
	<hr/>	
	Water balance .....	
NaCl balance .....		

## BLOOD CHEMISTRY

Date & Hour .....
Plasma K .....
Cell K .....
Whole blood K .....
Hematocrit .....
Plasma Prot. ....
Whole bld. vol. ....
Plasma vol. ....
Whole bld. S.G. ....
R.B.C. ....
H G B .....
Plasma Na .....
CO 2 content .....
Chloride .....
Urine S.G. ....
Urine alb. ....
Other .....
Other .....

R—Ringers S—Norm. Saline S.G.—Saline & Glucose  
H.S.—Hypertonic Salt G—Glucose T—Trans.

On December 13, 1940, a meeting was held to start this program. There were present:

Dr. Rhoads, <i>Chairman</i>	Dr. C. Medinger	Dr. E. Self
Dr. Drew	Mrs. Myron	Dr. W. Thalhimer
Mr. Folsom	Dr. J. Scudder	

Questions relating to the experimental set-up as proposed and approved by the Board of Medical Control at its meeting on November 27, 1940 were considered. It was decided:

1. That the first and most important question to be answered if possible is the relative toxicity of plasma and serum. To this end it was felt that the first two pools (8 liters each), one of serum and one of plasma, should be used for clinical trial after preliminary chemical, electrophoretic and biological tests, using every care possible to eliminate any unknowns such as pyrogen contaminated equipment, or fluids, unnecessary filters, or unnecessary clinical procedures during the period of transfusion.

2. That the pools should be numbered serially; those containing plasma to be designated as P1, P2, P3, etc.; those having serum to be likewise designated by S1, S2, S3, etc., so that it might be easy to trace at any time the source of the material in each pool and its eventual distribution.

3. That the final container for dispensing be a 500 cc. "Sterisol" ampoule. This container was chosen because it can be flamed closed at each end and thereby eliminate all corks, stoppers and gadgets as a possible source for untoward reactions. Further it was felt that the serum should be dispensed in 500 cc. lots, while the plasma should be dispensed in 550 cc. lots so that comparable amounts of the actual protein containing solution might be dispensed from each ampoule. In addition to this attempt to use comparable quantities in similar cases the protein content from each pool is to be actually determined in terms of grams per 100 cc. so that actual quantitative comparisons may be established.

4. In order to insure pyrogen-free equipment two suggestions were sustained:

- a. That Dr. Thalhimer should get in touch with Mr. Biehn of the Abbott Laboratories to find out if said Company would make a donation of sterile rubber tubing dispensing

outfits which have already been tested and found to be pyrogen-free.

- b. Dr. Scudder felt that, if such an arrangement could not be made whereby the apparatus shall have been tested before use, a conductivity bridge for making such tests might be purchased and the routines as established by Walters of the Peter Bent Brigham Hospital in Boston carried out.

5. Samples for tests should not be treated with Merthiolate before these tests are carried out because:

- a. The electrolyte content of the Merthiolate would greatly complicate the electrolyte studies.

- b. The amount of denaturation brought about by the combination of the mercury with the proteins is not known.

- c. Certain individuals have a particular sensitivity to mercury compounds and this would make more complex the problem of assaying the clinical reactions.

- d. In the biological tests the mercury compound may give false pictures in the exposed capillary field.

6. Samples necessary for the tests are as follows:

- a. For Tiselius Patterns—20 cc.

- b. For electrolyte studies—20 cc.

- c. For protein studies—20 cc.

- d. For perfusion experiments—200 cc. It was felt that in the first series tests of perfusion experiments should not be carried out.

It was decided that the samples should be uniformly delivered to the various laboratories from Dr. Thalhimer's laboratory in small pyrex bottles with ground glass stoppers hermetically sealed with cellophane tissue.

7. It was voted that none of the solutions in the first two pools would be used for drying.

8. Dr. Medinger stated that they now have on hand 17 flasks of plasma which had been dried by the method developed at the Memorial Hospital. This material is ready for clinical trial. Dr. Self is to use this material on suitable clinical cases as soon as the apparatus for introducing the sterile water with which the dried

plasma is to be reconstituted can be perfected by Mr. Folsom and Dr. Medinger. No tests are to be done on this batch of dried material. The object is simply to determine whether it may or may not be given safely and whether any changes in the technique of drying may prove to be necessary before the material now being prepared at Dr. Thalhimer's laboratory is sent back to Dr. Rhoads' laboratory for drying.

9. Dr. Self stated that for this series of experiments it seems wise not to use cases in severe shock, since minor reactions in the form of fever, chills, or urticaria, were not demonstrable in the presence of total circulatory collapse. This opinion was concurred with by all present.

Dr. Drew was to coordinate this research.

It is hoped that definite results may be available as a result of this approach to the problem by June, 1941. In spite of the urgency of the moment to supply some suitable blood substitute for the use of the armed forces of the United States Navy and Army, it is felt by this Association that every effort should be made to carry this work to a successful conclusion and thereby make available to other workers in this field information which may possibly guide their future activities along surer paths. It is felt that an aggregate amount of about 1,000 liters of plasma and serum will be required to complete these experiments on a large enough scale to be of statistical value.

#### A CENTRIFUGE OF THE CREAM SEPARATOR TYPE FOR THE SEPARATION OF BLOOD CELLS AND PLASMA

When reports were received that in one or two of the larger stockyard laboratories blood of cattle was separated into plasma and cells by means of centrifuges of the cream separator type, this method was investigated. Reports from England subsequently received indicated that a modification of such apparatus is being used there to separate human cells from plasma. The obvious advantage of this rapid separation of cells from the plasma is justification for the expenditures which have been made to obtain a DeLaval separator centrifuge, redesigned according to specifications drawn up by the Medical Supervisor, for the purpose of more rapidly, cheaply and safely obtaining large quantities of plasma. This machine should be delivered in February or early March. If it is capable

of doing all that the manufacturers claim it will do, that is, separating clear non-hemolyzed plasma from fresh human blood at the rate of 50 liters an hour, one of the largest problems in mass production of blood substitutes will have been solved.

#### BACTERIAL FILTRATION

Another very important piece of experimentation gotten under way by the Association is that of determining the practicability, the efficacy and flow rate of large Seitz Filters. Previous work had shown that it is practically impossible to filter plasma through Berkefeld or Chamberland filters. Fortuitously, at the time this part of the program received the sanction of the Board of Medical Control, the British Red Cross through the American Red Cross requested that this Association test out certain large American Seitz filters before shipment to England. This part of the experimental program was delegated to Dr. Unger, Dr. Thalhimer, Dr. Drew and Dr. Hans Clarke.

The Seitz filter pads are composed of asbestos fibers in a fine mesh of wood pulp. They act, not by simply filtering bacteria but rather, it is thought, in a more complex manner depending on physical chemical principles of adsorption.

Experiences at the Post Graduate Hospital, Presbyterian Hospital and at the Manhattan Serum Laboratory have shown that plasma filtered through one pad had a tendency to clot more rapidly than unfiltered plasma and plasma filtered through two filters, either consecutively or following an interval of time clots even faster.

This finding was a great disappointment. A cause was sought. The first supposition was that the citrate was removed by the filter pads, hence the subsequent clotting. This may be so but definite evidence has not yet been adduced to sustain this supposition.

Two findings have been made, however, which throw real light on this problem. The first is that a sample of plasma after passing through a single filter pad may contain 100% more calcium than the original sample and that this amount increases with each subsequent filtration. Secondly, the amount of fibrinogen decreases up to a point during the filtration through a single pad, and then increases up to the time when filtration ceases entirely as a result of complete plugging of the pores.

Dr. Unger is to make a detailed report of these findings in a separate communication. Dr. Hans Clarke, Professor of Biological



Chemistry of the College of Physicians and Surgeons, is likewise continuing his investigations along these lines with the hope of arriving at some conclusion.

The explanation seems to lie in the fact that the first filtrate which has passed through has most of its fibrinogen precipitated by or on the filter pad, and, since the small amount of calcium washed out in the early stages is not great, this portion of the filtrate does not clot. Later, however, as the process goes on, more and more calcium is washed out of the filter pads and these same pads, having absorbed nearly as much fibrin as they can, begin to allow greater quantities of the fibrinogen to pass through. This fibrinogen in the presence of markedly increased quantities of calcium causes its rapid precipitation so that a curve of expectancy may almost be calculated; the last portions clot soon, the middle fractions later while the first filtrate through may not clot at all.

It is strongly recommended that a central laboratory be set up, containing a blood separator-centrifuge from which a steady stream of plasma would run into a pressure chamber from which it could be forced by CO<sub>2</sub> through a clarifying filter and then a bacterial filter before dispensing into sterile final containers.

The suggestion was made by Dr. Earle Taylor that the breweries in this country used a filter of loose diatomaceous earth for clarifying beer. This was in December, 1940. In January, Dr. Philip Wilson, having just returned to America from service as Director of the American Hospital at Basingstoke, England, reported that the British authorities, having run into the same difficulties in filtering plasma, had turned to their breweries for this stage of the processing. The results apparently were encouraging but still definitely in the experimental stage. This method is now being given a trial in one of the local laboratories.

If it is not possible to obtain an adequate flow of bacterially filtered plasma, as a result of the various studies above referred to, the tendency would be to make serum the substitute of choice if sufficient evidence can be rapidly produced to show that it is a safe substance to give in repeated large quantities.

#### SUMMARY

(1) The dumb-bell bottle has faults which require correcting before it can be recommended as the donor container of choice, when plasma is to be siphoned from containers after spontaneous sedimentation has occurred.

(2) Repeated test runs have shown that centrifugation yields uniformly greater amounts of plasma than sedimentation.

(3) Separation of plasma from red blood cells by a modified cream separator centrifuge seems near accomplishment but has not been demonstrated.

(4) Bacterial filtration of plasma in large quantities has been found impracticable by any of the commonly used types of filters. This difficulty will be overcome in the near future it is hoped by the use of calcium free filter pads.

(5) Experimental evidence derived from the limited number of clinical trials on human beings, completed to date, is insufficient to justify recommending the widespread use of human serum as a blood substitute by this Association, but further work is in process.

(6) A method has been devised whereby either plasma or serum may be dried by a rather simple and cheap apparatus designed on the principle of the early "Lyophile" machines.

#### VII

##### Facilities for the Collection of Blood and the Preparation of Plasma in the Larger Cities

Mr. DeWitt Smith, of the American Red Cross, early in December, requested that this Association suggest a program which might be quickly carried out for the bleeding of 100,000 donors in six months, should this amount of blood be asked for by the United States Navy.

Before submitting any such plan, it was felt advisable to get first-hand information concerning the blood banks and plasma banks now in operation in the larger cities of the United States. It was thought that these would be the most logical centers around which to rapidly build a national organization. To that end, on December 20th, a letter was sent to key individuals in the following cities: <sup>1940</sup>

Boston	Chicago	St. Louis, Mo.
Philadelphia	Detroit	New Orleans
Baltimore	Cleveland	San Francisco
Washington, D. C.	Cincinnati	Los Angeles

in order to ascertain what facilities each of these cities offered which might be used at an early date for the collection of blood and the processing of plasma. To date answers have been received from the following:

*San Francisco*—Dr. John R. Upton, Secretary-Treasurer of the Blood Bank of the San Francisco County Medical Society, writes as follows:

"At the present moment there is only one small blood bank in San Francisco and that is out in the San Francisco County Hospital. In view of this, and due to certain physical factors which make enlargement of this bank somewhat difficult, approximately three months ago I cast about for a site which would be desirable for us to use in connection with our dried plasma project of the British War Relief Association. The site was found and we should have the plant working by the end of January. I had collected a rather large sum of money, enough to see me through my first year of running, when I heard that our San Francisco County Medical Society had appointed a committee of three to study blood banks, but due to lack of financial assistance no progress had been made. We joined forces, my cash and their rooms, and the result is we have engendered a lot of enthusiasm that only needed the spark. The San Francisco County Medical Society bank will run as a non-profit organization and we have appointed four top laboratory men to help us so that we can more than pass the strict requirements laid down by our Board of Health Department.

"The above two blood banks are the only two in San Francisco. One feeds the large County Hospital, the other will be run as a non-profit blood bank for San Francisco and the Bay Area. Naturally I shall be happy to cooperate with you to the fullest."

*Los Angeles*—Dr. P. Berman, Chief of the Medical Service of the Los Angeles County Hospital, summed up the facilities in Los Angeles as follows:

"To my knowledge the only blood bank operating in this area is the one located at the Los Angeles County Hospital. We take care of approximately 4800 blood transfusions a year and have equipment for drying of serum and plasma if necessary.

"There is a commercial laboratory in Los Angeles, The Hyland Laboratories, 4524 Sunset Blvd. (Dr. Clarence Michael Hyland), where plasma and serum are manufactured

for one of the pharmaceutical houses in California; but they do not operate a blood bank and do not sell blood for transfusion.

"Dr. A. M. Zeiler of the firm of Zeiler, Hammack, and Maner, 657 South Westlake, a commercial Laboratory in Los Angeles doing a great deal of blood transfusion work, states that they are equipped to prepare serum and plasma and take care of 100 donors a day on short notice."

*St. Louis, Mo.*—Dr. Evarts A. Graham, of the Washington University, writes as follows:

"I am inclined to think that the present facilities are not adequate but that they can be made so by arousing the interest of various groups of people here in the city. There is in existence a volunteer donors' organization. I am sending your letter to Dr. Frank Bradley, Superintendent of the Barnes Hospital and shall ask him to communicate with you directly."

*Chicago, Ill.*—Dr. Sidney O. Levinson, Director of the Samuel Deutsch Convalescent Serum Center, writes as follows:

"The Samuel Deutsch Serum Center at Michael Reese Hospital has engaged in serum and plasma preparation for general distribution both in the city and in the state. Our facilities are quite adequate to process several hundred units per week. This could undoubtedly be greatly amplified if there were funds to increase the equipment which is needed in this work.

"The only blood bank of any size or consideration in the City of Chicago is the one that is conducted at the Cook County Hospital. There are a few small blood banks in some of the smaller hospitals, and we stock a small supply of preserved blood, but I do not believe that any of them are of a size to warrant consideration for a program such as you are carrying out in New York."

We have been in communication with Dr. Schirmer of the Cook County Hospital but to date no answer has been received.

*New Orleans*—Dr. Alton Ochsner, of Tulane University, writes as follows:

"We have not had in New Orleans a blood or plasma bank but at the present time such is being started at the Charity Hospital. Dr. M. E. DeBakey will probably be in charge of it."

*Cincinnati*—Dr. Mont R. Reid, of the University of Cincinnati and Chairman of the Blood Transfusion Service, writes as follows:

"The Cincinnati Chapter of the American Red Cross is operating a very efficient blood transfusion service in this city. It is located at the Cincinnati General Hospital and is directed by Dr. Paul I. Hoxworth. I believe that it is the most efficiently run service of this type that I have seen. We have not yet gone into the preparation of wet and dried plasma but our plans are all drawn up for this purpose. I know of no place which has access to such a large volume of blood as we have here and I am sure that if the occasion arises we could cooperate most satisfactorily with the B.T.B.A."

*Washington, D. C.*—Dr. Edgar A. Bocock, Supt. of the Gallinger Municipal Hospital, writes as follows:

"According to available information blood or plasma banks are conducted in Washington by the following:

1. Gallinger Municipal Hospital
2. Providence Hospital (small plasma bank)
3. Emergency Hospital
4. Children's Hospital.

"The latter three are probably limited in extent.

"The bank at Gallinger Hospital has been in service actively for several years and in the past year has been transformed almost completely into a plasma bank, and that material is now being largely used in place of whole blood.

"I feel that with the already existing satisfactory nucleus on hand at this institution it would not be difficult to expand into a plasma station that could be readily made available for the collection and preparation of large quantities of this material.

"We have a staff of very well-trained individuals who probably have done as much or more along the lines of plasma utilization than any other similar group in the country. I refer to Drs. Charles Stanley White and Jacob Weinstein, who since its inception have been charged with the conduct of the blood bank here.

"I assure you that this institution stands ready to cooperate very fully with your organization and the American Red Cross in the event a movement is commenced looking toward the establishment of a collecting station in the Capital."

*Boston*—Dr. Elliott C. Cutler, of the Harvard Medical School, writes as follows:

"We have had in Boston recently a meeting concerning the establishment of a common blood bank and have agreed that at the major hospitals here blood will be collected and then forwarded to the State Antitoxin Laboratory for safe keeping and dissemination. The man in charge of this work at the Peter Bent Brigham Hospital is Dr. Carl W. Walter."

(Dr. Robinson is director of the Laboratory.)

*Baltimore*—Dr. Winford H. Smith, Director of the Johns Hopkins Hospital, writes as follows:

"There are only two blood banks in Baltimore, one at the University of Maryland Hospital and the other at the Johns Hopkins Hospital. Neither of these would be prepared to take on the work you suggest without additional equipment and apparatus. If, however, it were made possible for these institutions to install such additional equipment, I am sure that either or both of them would be glad to cooperate. The space for such a laboratory at the Hopkins is very limited. If it were desired to set this up on a fairly large scale I imagine the University of Maryland Hospital could handle it better because they have a new laboratory building with a good deal of space unallocated at the present time."

*Detroit*—Dr. Roy D. McClure, Surgeon-in-Chief of the Henry Ford Hospital, writes as follows:

"We would be tremendously interested here in helping in every way that we can with this work. I am enclosing you a reprint of Dr. Hartman's. Dr. Hartman is in charge of our laboratories. We are convinced that this is by far the quickest, and we believe the best, method of today. We have successfully used this dried blood plasma in our operating rooms for some months. At the present time there are three blood banks in Detroit in action. One is at the Receiving Hospital, one is at Harper Hospital and one at our own hospital, and they are establishing a blood bank at the Grace Hospital."

We have asked Dr. R. H. Bishop, Medical Director of the University Hospital in Cleveland; Dr. Elizabeth Helene Schirmer, of the Cook County Hospital in Chicago; and Dr. Aims C. Mc-

Guinness, Director of the Philadelphia Serum Exchange, for a report on the facilities in their respective cities and have no doubt they will be in at an early date. Their replies should make fairly complete the information on the facilities for immediate operation in the larger cities of the country. Since there are large medical centers in each of these places it is felt that they represent the centers most likely to be conversant with modern methods of collecting, storing and processing human blood.

## VIII

### Plan for Collecting 100,000 Bloods

With the information obtained as set forth above, and pursuant to the request of the Red Cross heretofore mentioned, a plan has been drawn up which would utilize facilities in ten cities for the collection of blood from 100,000 donors over a period of six months.

The work would be built around units capable of handling 500 bloods per week, that is 100 bloods a day for five days a week. At this rate 20,000 bloods could be collected each month for five months to make a total of 100,000.

This presupposes that instructions detailed enough to start each of these units into actual production could be supplied. Such detailed instructions are not available at this time, yet it is hoped that the work in New York by the Association, now in process and as above proposed, will make the information available at a very early date.

At the present time the ideal processing plant would seem to be a centralized unit capable of handling 100 bloods per day; capable of doing the necessary bacteriological studies, preparing the apparatus for re-use, typing the bloods, separating the cells from the plasma, pooling the plasma, clarifying the pools, and filtering before final dispensation into the containers for use in the liquid form or for drying by an acceptable method.

To the end of accomplishing as soon as possible the building of a model central laboratory here in New York, five steps have been taken:

(1) A DeLaval Centrifuge Milk Separator has been requested for trial and experimentation in order to discover whether a modification in it might be made which would make it usable as a separa-

tor of blood and plasma. This machine is to be supplied at no cost to the Association, except the minor cost of shipping charges. (This centrifuge is to be sent to the Memorial Hospital for testing by Dr. Rhoads and Mr. Folsom.)

(2) A DeLaval Blood Separator of the type now being used in one of the larger packing companies for the separation of cattle blood has been ordered, under a guarantee that it will separate human cells from human plasma without causing hemolysis. (This centrifuge is to be set up in the central laboratory under Dr. William Thalheimer's direction.)

(3) Through arrangements with the American Red Cross a No. 20 silver-plated Seitz Filter, ordered by the British Red Cross, has been delivered to this Association for testing in order that a report might be made to the American Red Cross and through it to the British Red Cross as to the advisability of purchasing 11 more. To date valuable information concerning this filter has been gathered and improvements have already been made to increase its efficiency.

(4) A block tin-plated No. 20 Seitz Filter has been ordered by this Association and set up as a part of the equipment necessary to complete the central laboratory set-up. This will be set up in conjunction with the blood separator.

(5) A pressure tank designed to feed plasma to the filters at a uniform rate under CO<sub>2</sub> pressure has been ordered.

It is hoped that after the combined use of these pieces of apparatus is established as a practical working unit the process recommended will be somewhat as follows:

(1) The collection of bloods in hospitals or other laboratories by a trained staff.

(2) The delivery of such bloods to the central laboratory by American Red Cross Ambulance Units, when the typing and serology reports have been made.

(3) The pooling of all bloods of like types in the central laboratory—that is, all A's will be pooled into one batch, all B's, etc. It is felt that this procedure is superior to that carried out by the English in their four plasma centers at this time. They pool all bloods in order that the plasma which is gotten will be free from

agglutinins, but it is practically impossible to pool whole bloods of different types without getting the production of isohemolysins and without some hemolysis.

(4) The pooled bloods to be run consecutively into a separator centrifuge.

(5) The separator centrifuge to be adjusted so that the cells will be thrown out of the lower bowl as a waste product while the clear plasma is thrown out of the upper outlet into a second separator centrifuge.

(6) The second separator centrifuge to be so regulated that the fat and fibrins will be whirled out of the upper opening while the somewhat clarified plasma will this time be discharged through the lower opening which is aseptically connected to a pooling tank.

(7) Collection of the plasma of all groups in such pooling tank for the purpose of suppressing the agglutinins.

This is a convenient place for a break in the series of steps but ideally this process should continue by forcing the plasma with CO<sub>2</sub> into—

(8) A clarifying Seitz filter and through this to

(9) A Seitz bacterial filter; then through

(10) A fine filter of diatomaceous earth or spun-glass to remove any possible shreds of asbestos before the final product is run into

(11) Dispensing bottles for shipment or containers which might be immediately attached to

(12) A drying apparatus for the preparation of dried plasma.

There is evidence from several sources both in this country and in England that such a set-up will work. It offers many advantages in the form of safety, speed and efficiency but must be built and proved to be practical before it could be recommended as the ideal set-up for use in other cities.

## IX

### Conclusions

These conclusions are necessarily incomplete because adequate reports have not been received from England concerning the effectiveness of the material sent; experiments begun for the purpose of establishing the most ideal set of containers for the blood and plasma have not been completed; experiments for determining the relative toxicity of plasma and serum in the liquid and dried forms have not reached the stage where accurate conclusions may be drawn; investigations related to the problem of delivering a uniformly and constantly uncontaminated end product are still in progress, and the establishment of a central laboratory constructed on the principle of separating the plasma from blood by means of a separator centrifuge and double filtration of all such plasma has reached the blueprint stage only. Much work is still needed before definitive data is available related to each of these problems.

Certain principles, however, can be stated with some conviction at this time. These may be recorded as follows:

(1) Plasma as produced by this organization and shipped to England is a safe blood substitute. It is the opinion of this body that evidence will grow to sustain the thought that either serum or plasma if properly prepared may be used with equal effectiveness and safety.

(2) Only healthy, adult donors should be used as a source of blood. For the protection of the donor, an adequate physical examination to rule out any organic defect or functional derangement, which might be the source of trouble following phlebotomy, should be done. For the protection of the future recipient, donors with communicable diseases or serological evidence of syphilis should not be used.

(3) Adequate records should be kept of every step of the procedure not only for the identification of specimens but in order that any individual blood, plasma, or collections of plasma may be traced rapidly through the whole process of collection and preparation.

(4) In collecting blood, regardless of the type of bottle used or the later processing of the plasma, a closed system should be established and maintained throughout.

(5) Whole blood should not be traumatized in any way such as by too much shaking to distribute anticoagulant, by storage in a vibrating refrigerator, or by transportation to points relatively far removed from the collection center. It would seem unwise to allow the blood to stand for a period exceeding a week before removing the plasma.

(6) Centrifugation is definitely established as preferable to sedimentation as a preliminary step to the collection of the plasma. A study of the comparative yields in the body of the report adequately sustains this conclusion.

(7) Pools of at least two liters (from eight donors) and preferably larger should be made of the plasma, before dispensing into final containers, in order to suppress specific agglutinins and thereby obviate the necessity for typing any of the material used.

(8) Some antiseptic should be used to prevent the multiplication during storage of any chance contaminant. "Merthiolate" (Lilly) in a dilution of 1:10,000 was used in this project. It is felt that the ideal preservative has not been found.

(9) The desirability of universal bacterial filtration for any material prepared for storage and later intravenous use has been firmly established, but the actual technique of filtering large quantities of plasma has not been perfected. Two chief difficulties remain: first, attaining sufficient speed of filtration to approach mass production methods; secondly, the problem of clotting in the plasma which has been filtered.

(10) Safety demands that careful bacteriological tests for the detection both of aerobic and anaerobic organisms be run on each pool of plasma and on one final sample container from each pool to insure sterility of the product. These tests must comply with the local, state and federal laws and regulations pertaining to the preparation of biologics.

(11) Biological tests should be carried out on animals in order to assure a non-toxic final product.

(12) Plasma and serum in dried form have many advantages over the liquid products. They are more easily stored, deteriorate more slowly, and chance bacterial contaminants will not grow in

the dried state under vacuum. It is felt, however, that a larger and more thoroughly controlled series of clinical trials should be reported before large quantities of these dried materials are released for general use. Operating efficiency and capital outlay are the principal determining factors in the choice of a drying apparatus, since there are no essential differences reported to date in the efficacy of the product produced by the various systems now advocated.

## X

## Bibliography

As a part of the program for gathering as much information as possible relating to the problems of plasma and serum preparation and preservation, a library of subjects related to this problem was begun and to date there are on file in the office of the Blood Transfusion Association, the following reprints:

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